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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-FIRST MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

MARCH 1-2, 1955

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Initial Distribution

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Twenty-first Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Council Chamber, National Research Building,
Ottawa, March 1 - 2, 1955.

1. Attendance

Dr. J.B. Macdonald, Chairman
Dr. J. Aldous
Dr. H.J. Barrie
Dr. J.M.R. Beveridge
Dr. H.K. Brown
Dr. A.C. Burton
Col. C.B.H. Climo (representing Brig. E.M. Wansbrough)
Dr. D.H. Copp
Dr. A.W. Ham
Dr. C.D. Husband
Dr. L.A. Kilburn
Dr. J.-P. Lussier
Dr. R.H. McDougall
Dr. E.G.D. Murray
Dr. E. Pagé
Dr. A.G. Racey
Dr. C.H.M. Williams

Dr. J.B. Marshall, Secretary
Miss D.J. Wright

By Invitation

Dr. L.F. Bélanger
Dr. E.M. Madlener
Dr. G. Nikiforuk
Dr. J.J. Rae

Apologies for his absence were received from:

Dr. J.S. Bagnall.

2. Chairman's Remarks

The CHAIRMAN welcomed to the Committee Dr. E.G.D. Murray, who had been designated by Council as its representative on the Associate Committee on Dental Research, Dr. H.J. Barrie and Dr. C.H.M. Williams, all of whom were attending their first meeting. He also extended a welcome to Col. Climo, representing Brig. Wansbrough whose return to Ottawa had been unavoidably delayed, and to the four invited guests, Dr. Bélanger, Dr. Rae, Dr. Nikiforuk and Dr. Madlener. He further expressed the hope that the guests would feel free to take part in the discussions of the Committee.

The CHAIRMAN reported that on the basis of the decision made at the 20th Meeting, a brief outline of the work of the Committee, and information regarding the research supported by the Committee, had been distributed to the basic science departments of Canadian Universities. He felt that the results of this action had been quite gratifying.

3. Minutes of the Twentieth Meeting

MOVED by Dr. McDougall and seconded by Dr. Copp
THAT the Minutes of the Twentieth meeting
be taken as read and approved.

CARRIED

4. Business Arising From the Minutes

(i) Fluoridation (Min. 6, 20th Mtg.)

The CHAIRMAN opened the discussion on the advisability of sponsoring a symposium on fluoridation under the auspices of, and paid for by, the Committee by pointing out that the situation had changed somewhat since the question was raised at the previous meeting: a larger number of applications for grants in aid of research for the coming year had been received, and the allocation of funds for a symposium might necessitate the rejection of worthwhile applications for grants. DR. MURRAY pointed out that Council would have to be consulted if the Committee considered holding a symposium since it would involve the policy regarding the use of public funds made available to the Council. He said that the Council

could not itself undertake to hold such a symposium but if the Committee wished to sponsor it, it might apply to Council for funds to be used in partial support of the symposium.

DR. BURTON raised the point of the Committee's responsibility in connection with matters of this kind, which are directly related to the field of dentistry. DR. NIKIFORUK mentioned that it would be of great value to the Canadian Dental Association to be able to add the name of the National Research Council to the list of those organizations supporting fluoridation.

DR. BARRIE pointed out the Committee could not express an opinion on the subject without first investigating the matter thoroughly. In reply to DR. BURTON's question as to whether the Committee had any obligation to initiate research on this subject, the CHAIRMAN stated that while it is the Committee's aim to promote dental research, it is unreasonable for the Committee to suggest what research be done; the projects supported should be initiated by the researchers on the basis of their interests.

The CHAIRMAN read to the meeting the terms of reference of the Committee; after considerable discussion it was agreed that, while the Committee might undertake an evaluation of research results at the request of an educational or governmental agency, it would be going beyond its jurisdiction if it were to make any recommendations regarding fluoridation. It was felt that such a subject falls more properly within the scope of the federal and provincial departments of health.

It was AGREED that no action would be taken to sponsor a symposium on fluoridation.

(ii) Special Travel Grants (Min. 7, 20 Mtg.)

The CHAIRMAN reported that the Executive had authorized travel grants to four individuals to attend the meeting of the International Association on Dental Research in Chicago in order to present reports on work sponsored by the Committee. These grants were made to Dr. Rae, Dr. Madlener, Dr. Hunter and Dr. Macdonald.

The CHAIRMAN drew the attention of the

Committee to the fact that particular care would have to be taken in future in awarding travel grants. He pointed out that the amounts requested for grants in aid of research would probably be greater than previously and for this reason he felt that the policy in respect to travel grants should be reviewed by the Committee.

It is the present policy to make travel grants only to those individuals who are invited to attend scientific meetings in order to report on work done under the auspices of the Committee or to individuals whose research projects require travel to other organizations. DR. MURRAY felt that the cost of attendance at meetings should be met either by the universities or by the individuals concerned, rather than by the Committee, if such grants would mean the reduction of funds allocated to research, but he agreed with DR. BURTON that there were cases in which funds could better be spent on travel if it was the opinion of the Committee that a researcher could gain considerably from personal contact with another expert in the field.

After some discussion, it was MOVED by Dr. Copp and SECONDED by Dr. Aldous

THAT every request for funds, including funds for travel, be considered on its own merits in relation to the funds available and other requests received.

CARRIED.

An amendment was PROPOSED by Dr. Beveridge and SECONDED by Dr. Ham.

THAT requests for funds to support research projects, travel essential to the prosecution of these projects, and fellowships be given first consideration, and that requests for funds for travel not essential to the prosecution of the research projects be considered last.

NOT CARRIED.

(iii) Reprint Costs (Min. 11, 20th Mtg.)

The CHAIRMAN read to the meeting a letter

received from the President following Council's consideration of the recommendation that the cost of reprints of papers published on work supported by the Committee be borne by the Council. The recommendation was rejected by Council primarily because of the considerable expense which would result from an overall application of this procedure. The CHAIRMAN also read his reply to the President, in which he drew attention to the fact that publication was a necessary part of research and the question had been raised because some universities would not pay the cost of reprints required by the authors.

DR. MURRAY pointed out that while publication is an essential adjunct to research, the purchase and distribution of reprints could only be regarded as a matter of personal convenience which was not necessary; Council could not therefore consider the cost of reprints as a legitimate charge against a grant.

DR. ALDOUS stated that the cost of reprints was usually relatively small but if publication of a paper requires the reproduction of plates the cost of publication is quite high. Such a charge is related to the actual publication of research results rather than to reprints. The SECRETARY felt that Council might view with more sympathy a request for payment of the cost of essential plates, although in view of its present subsidization of Canadian research journals it might prefer to have the paper containing such material published in one of its own journals and absorb the cost in that way.

(iv) Preparation of Reviews Under the Auspices of the Committee

The CHAIRMAN noted that this subject had been discussed very briefly at the last meeting and it was felt that grants in aid might be made to individuals undertaking to prepare a review.

DR. MURRAY remarked that if such aid were given, the resulting publication would normally become a Council publication.

The CHAIRMAN suggested that this question be left in abeyance and if a request for aid is received from

someone wishing to prepare a review of interest to the Committee, it could then be considered on its merits.

5. Consideration of Reports from Grantees

At the meeting, the reports from grantees presenting their reports in person were considered first, and other reports were presented as usual by members of the Committee as requested by the Chairman. For convenience of reference in the Proceedings, however, the reports are arranged in numerical order by project number. During presentation of the reports on his own two projects, DR-35 and DR-50, the Chairman asked Dr. Racey to take the chair.

i) DR-1: Dr. J.J. Rae
Reported by himself

"Chemical Mechanisms in Dental Caries"

A summary and progress report appear in APP. E.

Dr. Rae reported on the three phases of his work: 1) ammonia production in saliva, 2) lactic acid production by saliva, and 3) a bactericidal substance prepared from tooth enamel.

In connection with the production of ammonia in saliva, Dr. Rae reported that results showed that the addition of urea to saliva increased ammonia production, the addition of glucose inhibited ammonia production and the addition of a mixture of glucose and urea gave somewhat confusing results but appeared to enhance ammonia production. Four papers have been prepared on this phase of the work and it is now regarded as having been completed.

DR. RAE also reported that work on lactic acid production by saliva has now been concluded, since he had found that no caries index could be obtained from lactic acid measurements because of the presence of other bacteria. To DR. MURRAY's question as to the length of time during which the saliva-glucose mixtures were incubated, DR. RAE replied 18 - 24 hours. It was suggested that a longer period might have been advisable; the CHAIRMAN said that a correlation between caries activity and pH could be found after 48 hours' incubation.

DR. NIKIFORUK was asked to reply to a

question regarding the amount of urea in saliva; he said that in view of the physical properties of the urea molecule one would expect that it might readily diffuse; the amount of urea in saliva is strongly correlated with its concentration in the blood; the average concentration of urea in saliva in 7-year-old children is 13mgs.% and considerably higher in the case of adults. He added that the ammonia level in saliva is not in any way correlated with urea in saliva.

DR. RAE explained that work was proceeding on the separation and identification of a substance in tooth enamel which appears to inhibit the growth of Lactobacillus acidophilus. It seems to be a halogen-containing organic substance, and paper chromatography and other organic analytical procedures are being used in its investigation. He also stated that he was interested in the problem of urea in food; he was proposing to study the effect of adding urea to a glucose-containing food such as milk chocolate in an effort to determine whether it would cut down the rate at which acid production takes place.

After further discussion of Dr. Rae's findings and of the relation of his work to the problem of dental caries, the CHAIRMAN thanked Dr. Rae for attending the meeting and presenting his report.

(ii) DR-13: Dr. M. Doreen Smith
Reported by Dr. Page

"Fluoride content of dentin and enamel of teeth from Sarnia, Brantford and Stratford".

A report appears in APPENDIX J

After presenting Dr. Smith's report, Dr. Page said that in his opinion very little further information could be derived from the work without a statistical analysis of her findings to date. He noted too that correspondence with Dr. Smith appeared to indicate that she was somewhat reluctant to have such an analysis made since her results appeared to vary as her operators changed. The Committee felt that this indicated some systematic error in techniques which should be investigated. DR. PAGE also felt that the analysis made by Dr. Smith of the various nutritional groups was rather superficial and therefore lacking in significance.

The CHAIRMAN said that Dr. Smith has made

analyses of some hundreds of teeth since 1948 and agreed with DR. PAGE's suggestion that a statistical analysis of her findings was in order.

DR. BROWN said that Dr. Smith had indeed analysed a large number of teeth, but he also pointed out that Brantford water had been fluoridated only since June 1945; he said that Dr. Smith had obtained a sufficiently large number of teeth calcified prior to fluoridation but only a limited number of teeth completely calcified subsequent to fluoridation. The number of extracted teeth supplied to Dr. Smith fluctuates from 6 to 60 a year and were very difficult to obtain.

DR. BURTON suggested that since the problem was basic, rather than clinical in nature, animals might be used. DR. COPP replied that an extensive study has already been made on animals.

DR. COPP was of the opinion that no further analyses should be made of teeth until apparent systematic errors can be controlled.

MOVED by Dr. Copp and SECONDED by Dr. Aldous

THAT a qualified person be appointed to visit Dr. Smith's laboratory to discuss with her the methods ~~she~~ is using and the reliability of the fluoridation data she has collected.

CARRIED.

(iii) DR-23: Dr. C.P. Leblond
Reported by Dr. Burton

"Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements".

A report appears in APPENDIX H

After presenting the report, DR. BURTON said that he had every confidence in the work done and that a great deal had obviously been accomplished during the year. He remarked that he was impressed by the grantee's realization of the difficulties of quantitative interpretation of radioactive studies.

DR. BURTON wondered if adult animals could

be used more easily, but DR. COPP pointed out that it was easier to study young animals whose teeth were in the process of formation and added that sectioning is also much easier in the case of young animals.

The Committee felt that this project represented a fundamental approach to the formation of the tooth matrix which was of great importance.

It was AGREED that Dr. Leblond be invited to attend the next meeting to report on his work.

(iv) DR-28: Dr. O.J. Walker
Reported by Dr. Beveridge

"Investigation of methods for destroying the organic matter in teeth, bones, and other materials prior to determination of fluorine content."

A report on the project appears in APPENDIX M

DR. BEVERIDGE pointed out that no progress had been made during the year, and DR. HUSBAND explained the details of the difficulties encountered by the grantee in connection with the repair of his equipment.

The CHAIRMAN asked if the Committee felt that there was need to further the development of this particular method. DR. BEVERIDGE was of the opinion that in view of the difficulties other investigators were having in determining fluoride the Committee should be interested in the development of this rapid method for doing so.

DR. NIKIFORUK stated that a report had been presented by Dr. Armstrong at the 1954 meeting of the I.A.D.R. on a method for the analysis of fluoride based on distillation of hydrofluoric acid which was also suitable for the analysis of hard tissues. He suggested that this report be brought to Dr. Walker's attention. In answer to DR. PAGE's question as to whether Armstrong's method called for preliminary ashing, DR. NIKIFORUK said that it did not.

(v) DR-35: Dr. J.B. Macdonald
Reported by himself

"Bacteriology of periodontal disease. I. Experimental fusospirochetal infections with mixtures of pure cultures."

A summary and progress report appear in APPENDIX B

Dr. Macdonald reported that a temporary terminal point had been reached in connection with this project, pending the development of more suitable methods for in vitro and in vivo investigations, and no grant is being requested for the coming year.

He outlined the work done on fusospirochetal infection transmitted to guinea pigs and the recent attempts to develop suitable quantitative techniques for the study of exudates. Dr. Macdonald intends to continue to study this mixed infection.

(vi) DR-37: Dr. D.H. Jenkins
Reported by Dr. Racey

"Continuing studies in electromyographic techniques".

A summary of the work appears in APPENDIX D

After presentation of the report, there was some discussion as to the lack of detail given by the grantee; many members found the report obscure.

DR. MURRAY pointed out that in the past grantees had been asked to condense their reports to contain only the bare essentials.

Following consideration of this project, it was the general feeling of the Committee that there should be an orthodontist among the membership who would be well qualified to evaluate work of this type. It was also suggested that the opinion of an outside referee might be sought in such cases as it seemed advisable. DR. HAM said that the National Cancer Institute had found such a procedure very valuable.

(vii) DR-43: Drs. S.G. Davis and H.R. MacLean
Reported by Dr. Husband

"Electropotentials caused by dental alloys".

A summary of the work done appears in
APPENDIX I

DR. HUSBAND reported that the grantees are working on the hypothesis that the electropotential

in the mouth causes degeneration of dental pulp over a period of time and an attempt is being made to discover suitable insulators. They are now working on gold alloys.

DR. BURTON said that this project, bearing on direct current potentials and their effect on the body, is very interesting and potentially a very useful study. He felt that it was perhaps not being pursued sufficiently vigorously, but DR. HUSBAND pointed out that heavy University schedules left the grantees very little time for research.

DR. HUSBAND advised the Committee that both Dr. Davis and Dr. MacLean were going on sabbatical leave for a year but they hoped to continue work on this project after their return. The CHAIRMAN suggested that the present grant be terminated and a new application for aid be submitted when the grantees were in a position to continue the work.

It was AGREED that a full report on the results to date be requested of the grantees and distributed to the members of the Committee.

(viii)

DR-50: Dr. J.B. Macdonald
Reported by himself

"The cultivation and characteristics of Bacteroides nigrescens".

A summary and report appear in APPENDIX C

DR. MACDONALD reported on his studies on Bacteroides nigrescens and explained that the original project had developed into a consideration of the growth factor required for certain strains of the organism.

He stated that before any degree of success can be attained in the project a suitable assay method must be developed. No grant is being requested for the coming year.

(ix)

DR-51: Dr. R.L. deC H. Saunders
Reported by Dr. Ham.

"Study of human dental pulp blood vessels by X-ray microarteriographic methods".

A summary of the work appears in APPENDIX L

DR. HAM reported that this project is of considerable importance because the vascular pattern of the teeth has never really been delineated. He felt that Dr. Saunders was to be congratulated on his pioneering in this field of study.

DR. ALDOUS mentioned that the grantee has had difficulty in getting a microfocus unit with the resolution he needs for his study of the problem; he has made an exhaustive study of equipment available and has had tests run by various companies in an effort to discover the most appropriate unit for his work. On the basis of his investigations, he now hopes to be able to obtain a Hilger-Watt unit with a resolution of 1 - 5 microns.

(x) DR-52: Dr. L.F. Bélanger,
Reported by himself.

"Mechanism of bone and tooth formation in the light of autoradiography and histochemistry".

A summary of the work appears in APPENDIX A

During the course of his report on the progressive distribution of radioactive substances injected in young rats, DR. BELANGER showed a series of slides illustrating the absorption of S^{35} and Ca^{45} in various hard and soft tissues. It appeared that the mucous neck cells of the fundic gland were the site of the greatest absorption of S^{35} . The autoradiographs indicated that what is an intercellular reaction 4 hours after injection gives place to diffusion 4 days after injection.

In referring to the absorption of radioactive substances by the teeth, Dr. Bélanger showed that S^{35} appears very strongly in the area of new enamel and appears to migrate toward the youngest part. Very little S^{35} is found at the level of the dentin after short exposure but it is clearly defined after longer exposure. In reply to DR. COPP's question, Dr. Bélanger said that S^{35} in teeth must be related to organic matter since pictures similar to those shown have been obtained even after demineralization.

DR. BEVERIDGE inquired as to the length of

time between injection of S³⁵ methionine and sacrificing of the rats. Dr. Bélanger replied that the animals were sacrificed 1, 2, 6, 12 and 24 hours after injection.

After some further discussion, the CHAIRMAN thanked Dr. Bélanger for his very clear presentation and congratulated him on the excellent autoradiographs shown to the Committee.

(xi) DR-54: Dr. B.N. Kropp
Reported by Dr. Lussier

"Development of a rapid method for grinding thin sections of plastic-embedded teeth".

A report appears in APPENDIX F.

In presenting the report, DR. LUSSIER noted that the wheels of the machine had been changed to enable the operator to get a better section and eliminate the use of abrasive powder.

It was pointed out that such a machine should be useful in the preparation of class material, but that Norse of the Argonne Laboratory has developed a method for cutting sections of 5 - 10 microns which are much more useful for research purposes. DR. BARRIE felt that Dr. Kropp's machine might have possible applications in pathology.

DR. PAGE inquired if any thought had been given to the possible patentability of this machine. DR. BEVERIDGE reported that the matter had been discussed with the Patent Committee at Queen's but it had been their feeling that no novelty was involved; the Committee agreed with this view.

The CHAIRMAN said that the present submission could therefore be regarded as a final report on the project.

(xii) DR-55: Dr. M.R. Culbert,
Report by Dr. Copp

"A cephalometric study of maxillary growth as related to statural growth".

A summary of the work appears in APPENDIX N

DR. COPP felt that the report submitted was too short to permit the formulation of any opinion on the work. He felt, too, that the grantee should have attempted to analyse some of his results.

MOVED by Dr. Copp and SECONDED by Dr. Beveridge

THAT a full report be requested on this project for the next meeting.

CARRIED.

(xiii) DR-56: Dr. D.J.E. Mitchell
Reported by Dr. Brown

"Comparative study of palatal height, width and length in ancient and modern crania".

A summary of the work appears in APPENDIX K

DR. BROWN was of the opinion that only an orthodontist could give a review on the work being done on this project and recommended the appointment to the Committee of a person trained in this field.

The CHAIRMAN reported that Dr. Mitchell was at present visiting the Smithsonian Institute to gather further material in connection with this study, and said that the grantee would be asked for a full report for presentation at the next meeting.

(xiv) DR-57: Dr. G. Nikiforuk
Reported by himself

"The amino acid composition of enamel protein using paper chromatography".

A summary and report on the work appear in APPENDIX G

DR. NIKIFORUK reported on his project designed to study the organic and protein portions of the enamel of teeth by paper chromatography. Slides were shown to illustrate the method used and chromatograms were circulated to show the results obtained. Dr. Nikiforuk said that he was particularly interested in the soluble fraction of the dialysate of tooth enamel.

Dr. Nikiforuk demonstrated chromatograms of impurities traced to the dialysis sac. Extensive washing was not successful in completely eliminating this pattern when using an anhydrous spray; he felt that one of the impurities might be an actual component of the dialysis sac employed. For this reason it has not been possible to attribute the spots on the chromatograms to the soluble components of tooth enamel.

DR. BEVERIDGE questioned the high temperature for drying the teeth. Dr. Nikiforuk said that a lower temperature would be preferable but the higher one was used to save time as it facilitated the removal of the enamel from the dentin. He felt that there was little likelihood of a denaturing of the materials at this temperature since they are bound in calcified matter. DR. BEVERIDGE asked if drying the materials in vacuo with P_2O_5 had been tried. Dr. Nikiforuk replied that this had not been done as yet but that cold treatment should be tried.

DR. BEVERIDGE asked what the procedure for hydrolysis was. Dr. Nikiforuk reported that the Block and Bolling method was used; the criteria were a maximum amount of amino acid nitrogen vs. time for hydrolysis.

There was some discussion of the type of sac used and DR. PAGE asked if the unidentifiable spots on the chromatograms might be due to an impurity in a single batch of the sacs used. Dr. Nikiforuk said that three different batches had been used to date, with similar results. DR. RAE suggested the use of electro-dialysis in order to speed up the process and cut down the amount of material picked up by the sac, but Dr. Nikiforuk felt that this procedure would introduce still another undesirable factor into the study.

The Chairman thanked Dr. Nikiforuk for his presentation of the work being done on this project.

6. Report from Fellowship Holder

The CHAIRMAN invited Dr. Madlener, who was awarded a fellowship by the Committee for the year 1954-55, to report on his recent studies on Spirillum sputigenum, carried out at the University of Toronto under the supervision of Dr. Macdonald.

These motile cells, which occur in material

in the gingival crevice, were first observed in 1886 by Miller. Various strains of this organism have been isolated but cultivation and isolation are extremely difficult to achieve and cultures do not keep well.

Dr. Madlener outlined a recent review published by Lessel and Breed in "Bacteriological Reviews" in which it was suggested that because of the flagellation and type of motility Spirillum sputigenum might be grouped with Selenomonas and Selenomonastix in the genus Selenomonas. Dr. Madlener felt that such a classification was not justified and described the work he had done on the isolation of Selenomonas and of Spirillum sputigenum in support of his theory. This work has demonstrated that there is as yet no justification for including the three organisms in one genus.

After a discussion of the report and of the manner in which some of the difficulties might be overcome, the CHAIRMAN thanked Dr. Madlener for his presentation of the report on his work.

7. Finances

(1) Financial Status of Committee

At the request of the Chairman, the SECRETARY presented the financial statement of the Committee as of 28 February 1955:

Allotment	40,700.00
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Expenditures

Awards - Grants	23,645.00
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Fellowships	<u>6,000.00</u>
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	29,645.00
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Miscellaneous

Travel	1,689.05
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Duplic.	142.25
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Reprints	17.14
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Cafeteria	<u>17.95</u>
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	<u>1,866.39</u>
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	31,511.39
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Unencumbered allotment	<u>9,188.61</u>	40,700.00
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It was noted that after the cost of the 21st Meeting had been met there would be an unexpended balance of approximately \$7,800.

(ii) Estimates for 1956-57

The CHAIRMAN pointed out that estimates for the fiscal year 1956-57 should be considered since they were required by the Council before any further meeting of the Committee would be held.

In the course of the discussion, DR. HAM pointed out that except for the fact that funds remaining from the present year's allotment were available for capital grants towards the purchase of equipment necessary for the prosecution of research authorized by the Committee, the work of the grantees would be considerably curtailed. He felt that an increased amount might reasonably be requested for 1956-57.

DR. COPP stated that the opening of the proposed Dental School at the University of British Columbia would undoubtedly give rise to a further increase in the number of requests received for funds in support of worthwhile research in the field of dentistry.

MOVED by Dr. Copp and SECONDED by Dr. Lussier
THAT the Associate Committee on Dental Research
request an allocation of \$48,000 for the fiscal
year 1956-57.

CARRIED.

8. Applications for Grants in Aid of Research and Fellowships.

The CHAIRMAN reported that the Executive had given very careful consideration to all applications at its meeting on the evening of March 1, and its recommendations were presented to the meeting for discussion. Each case was then dealt with individually.

Grants in Aid of Research

(i) Renewals

DR-1: Dr. J.J. Rae -
Amount requested: \$1,080.

"Bactericidal material tooth enamel; urea
in food".

MOVED by Dr. Pagé, SECONDED by Dr. Burton.

THAT \$540 be granted to enable the grantee to complete his work on bactericidal material in tooth enamel, and that no support be given toward the investigation of urea in food.

CARRIED.

DR-13: Dr. M. Doreen Smith -
Amount requested: \$2,540.

"Fluoride content of dentin and enamel of teeth from Sarnia, Brantford and Stratford, and a statistical study of the data collected".

It was the opinion of the Committee that Dr. Smith's work to date had been important and that a statistical analysis of the results obtained would yield much useful information regarding fluoride in teeth. The Committee agreed that a qualified referee be appointed by the Chairman to visit Dr. Smith's laboratory to evaluate the project from a technical standpoint. In view of the difficulty of obtaining teeth samples for analysis, it was agreed that this part of the work might be held in abeyance pending the accumulation of more teeth.

MOVED by Dr. Burton and SECONDED by Dr. Lussier.

THAT \$1,000 be encumbered to permit a statistical analysis of the data obtained thus far, for release by the Executive provided the report from the referee indicates that such a statistical analysis would be worthwhile.

CARRIED.

DR-22: Dr. C.H. Best -
Amount requested \$4,475.

"Mechanism of repair of supporting structures of the teeth".

MOVED by Dr. Burton and SECONDED by Dr. Murray

THAT amount requested, less \$275 requested for workmen's compensation and employee's contribution to pension fund, be granted pending a decision by Council on the principle of payments of charges connected with the benefits noted.

CARRIED.

DR-23: Dr. C.P. Leblond -
Amount requested: \$3,500.

"Entry of phosphate, carbonate and calcium ions into teeth."

MOVED by Dr. Copp and SECONDED by Dr. Ham

THAT application be approved, with the deletion of the amount of \$150. requested for payment of Dr. Weiss.

CARRIED.

DR-28: Dr. O.J. Walker -
Amount requested: \$1,500.

"Investigation of methods for destroying organic matter in teeth prior to determination of fluorine content".

MOVED by Dr. Burton and SECONDED by Dr. Husband

THAT a grant of \$900 for 1955-56 and a capital grant of \$600 from 1954-55 funds be made to Dr. Walker with the understanding that this is to be a terminal grant since the Committee is anxious to have the project completed during the coming year.

CARRIED.

DR-37: Dr. D.H. Jenkins -
Amount requested: \$2,350.

"Studies in electromyographic techniques".

In considering this report, the Committee felt that the item for a secretary's salary could not be approved.

MOVED by Dr. Williams and SECONDED by Dr. Racey

THAT a capital grant of \$2,100 from 1954-55 funds, and a grant of \$200 for 1955-56 be made for this project.

CARRIED

DR-51: Dr. R.L. deC. H. Saunders -
Amount requested: \$6,408.

"Study of human dental pulp vessels".

In the course of the discussion of this project, it was noted that the grantee had applied for a capital grant for the purchase of a Hilger-Watt microfocus X-ray unit with a resolution of 1 - 5 microns. The SECRETARY reported that the Medical Committee had given its approval in principle for the application of funds remaining in Dr. Saunders' present grant MP-403 towards the purchase of this unit.

MOVED by Dr. Copp, and SECONDED by Dr. Page

THAT a capital grant of \$5,000 be made from 1954-55 funds for the purchase of a Hilger-Watt unit and accessories, and that a grant of \$1,400 for 1955-56 be made.

CARRIED.

DR-52: Dr. L.F. Bélanger -
Amount requested: \$2,040.

"Mechanism of bone and tooth formation".

MOVED by Dr. Copp, and SECONDED by Dr. Aldous

THAT the requested amount be granted.

CARRIED.

DR-57: Dr. G. Nikiforuk -
Amount requested: \$3,160.

"The amino acid composition of enamel protein using paper chromatography".

MOVED by Dr. Aldous and SECONDED by Dr. Beveridge.

THAT the application be approved

CARRIED.

(ii) New Applications

Dr. J.G. Aldous -

Amount requested: \$2,430.

"The metabolism of procaine hydrochloride and related compounds. II Diethylaminoethyl-4-amino-2-propoxy benzoate (Ravocaine) hydrochloride".

MOVED by Dr. Copp and SECONDED by Dr. Beveridge

THAT the requested amount of \$2,430 be allocated to this project.

CARRIED.

Dr. D.H. Copp -

Amount requested: \$3,680.

"Phosphorus deficiency: effect on bones and teeth".

MOVED by Dr. Ham and SECONDED by Dr. Murray

THAT the application be approved

CARRIED.

Dr. F. Clarke Fraser -

Amount requested: \$3,350.

"Studies on the pathogenesis of cortisone-induced cleft palate in mice".

It was reported that work has been going on for some time under the aegis of other committees. It was the opinion of the Committee that in relation to the work proposed, 2 graduate assistants seemed excessive.

MOVED by Dr. Murray and SECONDED by Dr. Ham.

THAT \$1,850 be allocated for this project, with the understanding that this be considered a terminal grant on this investigation.

CARRIED.

Dr. S. Kirkwood -
Amount requested: \$4,000.

"Role of the salivary glands in the extrathyroidal metabolism of the thyroid hormone".

It was the feeling of the Committee that the applicant should be more specific regarding the details of the proposed research, and also that the amount requested for supplies should be reduced.

MOVED by Dr. Ham and SECONDED by Dr. Beveridge.

THAT \$3,200 be encumbered for this project, pending receipt of further information from the applicant, and that the Executive be empowered to approve a grant of this amount if satisfactory clarification is received.

CARRIED.

Dr. N.F. Walker -
Amount requested: \$5,700.

"An evaluation of hereditary factors in certain characteristics of cranial and facial skeletal growth".

After careful consideration, the Committee felt that any available twin sample would not be sufficiently large to make possible the detection of any significant inheritance values; it was therefore MOVED by Dr. Copp and SECONDED by Dr. Murray

THAT the application be refused.

CARRIED.

Dr. A.S.V. Burgen -
Amount requested: \$3,400.

"The secretion of protein in the saliva".

Dr. Copp pointed out that the proposed project was supplementary to a project supported by the Medical Committee. Considerable discussion ensued regarding

the nature of the proposed research, after which it was MOVED by Dr. Beveridge and SECONDED by Dr. McDougall

THAT the application be referred back to Dr. Burgen with the statement that insufficient information had been given regarding the proposed research to enable the Committee to judge whether it should be supported.

NOT CARRIED.

After further discussion, it was MOVED by Dr. Copp and SECONDED by Dr. Lussier

THAT the amount of \$3,400 be encumbered, to be released for this project at the discretion of the Executive, if satisfactory clarification of the application has been received from Dr. Burgen.

CARRIED.

In connection with this application, the Committee AGREED with DR. HAM that Dr. Beveridge, Dr. Copp and Dr. Burton should also review any revised application received from Dr. Burgen.

Application for Fellowships

- (i) Dr. E.M. Madlener,
20 Sussex Avenue,
Toronto 5, Ontario.

The CHAIRMAN reported that Dr. Madlener, the recipient of a Fellowship from 1954-55, expects to complete the requirements for his Master's degree in the Fall and plans to apply for a Ph.D. program which will undoubtedly be approved in view of Dr. Madlener's excellent record. It was noted that Dr. Madlener intends to make a career of research and teaching in the field of dental bacteriology.

MOVED by Dr. Williams and SECONDED by Dr. Husband.

THAT a fellowship in the amount of \$3,000 be awarded to Dr. E.M. Madlener for the year 1955-56.

CARRIED.

- (ii) Dr. Michael Weiss
1832 Sherbrooke St. W., Apt. 5,
Montreal, P.Q.

Dr. Weiss is a Canadian, 31 years of age, who obtained his B.Sc. degree from McGill in 1952 and his D.D.S. degree from McGill in 1954. He intends to carry on research on the factors controlling the composition of saliva, under the supervision of Dr. A.S.V. Burgen of McGill University; he will be working toward the degree of M.Sc. in Physiology.

Dr. Weiss was recommended as an enthusiastic worker with proven research ability by Dr. C.P. Leblond, with whom he has worked, and by Dr. Burgen.

MOVED by Dr. Pagé and SECONDED by Dr. Lussier.

THAT a fellowship in the amount of \$3,000 be awarded to Dr. Michael Weiss for the year 1955-56.

CARRIED.

In connection with this application, the CHAIRMAN pointed out that the application was significant in view of the fact that very little dental research had thus far been carried out at the McGill Dental School.

Summary of allocation of funds for research and fellowships for 1955-56.

<u>Grants</u>	<u>1955-56</u>	<u>Capital grants (1954-55 funds)</u>
<u>Renewals</u>		
DR-1 Dr. Rae	\$ 540.	
DR-13 Dr. Smith	1,000.	
DR-22 Dr. Best	4,200.	
DR-23 Dr. Leblond	3,350.	
DR-28 Dr. Walker	900.	\$ 600.
DR-37 Dr. Jenkins	200.	2,100.
DR-51 Dr. Saunders	1,400.	5,000.
DR-52 Dr. Bélanger	2,040.	
DR-57 Dr. Nikiforuk	3,160.	
<u>New</u>		
DR-58 Dr. Aldous	2,430.	
DR-59 Dr. Copp	3,680.	
DR-60 Dr. Fraser	1,850.	

Deferred

Dr. Burgen	\$3,400.
Dr. Kirkwood	3,200.

Fellowships

Dr. Madlener	3,000.
Dr. Weiss	3,000.

To summarize:

	No.	Total
Capital grants approved from 1954-55 funds:	<u>3</u>	\$ <u>7,700.</u>

Applications approved for 1955-56:

Renewal of existing grants:	9	16,790.
New applications approved	3	7,960.
New applications deferred	2	6,600.
Fellowships approved	2	6,000.

\$37,350.

9. Application Form

In connection with the review of applications received by the Committee, there was considerable discussion of the form and nature of the application. It was the general feeling of the Committee that applicants should be encouraged to define clearly their proposed research and to provide the Committee with sufficient background information in the form of related literature references to enable the members to determine the probable value of the work which it was being asked to support.

DR. ALDOUS suggested that the same form as that used by the Medical Committee, which provides a full page on which the applicant can outline his proposed research, might be adopted by the Dental Committee.

DR. HAM mentioned that it was difficult to use the same form for first applications as is used for renewal applications, since the same degree of detail is not usually necessary in both cases.

DR. COPP suggested that only in the case of new

applications would the second page, as supplied at present with the Medical but not the Dental, application, have to be filled out.

DR. BURTON felt that the applicant should also be encouraged to state the bearing of his proposed research on matters of interest to the Committee to which he is applying for support.

It was AGREED that a revised application form be developed during the coming summer, along the lines of the discussion, and a draft distributed to the members of the Committee for their comments or approval.

DR. HAM further suggested that if, in the opinion of the Executive after their preliminary review of applications further information is required, the applicant be requested to supply the additional information before the application is considered by the Committee as a whole. It was AGREED that this would be desirable, and would in future be done.

10. Application for a Special Travel Grant

The CHAIRMAN read to the meeting a letter from Dr. L.F. Bélanger requesting a special travel grant of \$150. to assist in defraying the expense of attendance at forthcoming meetings of the Ciba Foundation in London and the International Congress of Anatomy in Paris, at which he had been invited to present reports on the work he has been doing under the auspices of the Associate Committee on Dental Research.

MOVED by Dr. Burton and SECONDED by Dr. Copp

THAT ~~the~~ amount of \$150. be granted to Dr. Bélanger for this purpose.

CARRIED.

11. Membership of the Committee

The CHAIRMAN drew the attention of the meeting to the rules governing membership on the Committee, which state that no member may remain on the Committee for more than two consecutive three-year terms without a break in his service on the Committee prior to any proposed re-appointment. He noted that the present terms of Dr. McDougall, Dr. Bagnall and Dr. Pagé would expire at the

end of March 1955; under the existing regulations Dr. McDougall would no longer be eligible for re-appointment at this time.

Consideration was given to the appointment of new members, during the course of which the CHAIRMAN pointed out the advisability of maintaining a geographical representation, as well as a group representation.

MOVED by Dr. McDougall and SECONDED by Dr. Copp

THAT Dr. Richard Cline be nominated to succeed Dr. McDougall on the Committee.

MOVED by Dr. Kilburn and SECONDED by Dr. Husband

THAT nominations for the replacement of Dr. McDougall be closed.

CARRIED.

In connection with the appointment of Dr. Cline, Dr. McDougall pointed out that his election was particularly suitable at this time, not only because of the opening of a new Dental School at the University of British Columbia, but also because the Committee appeared to be agreed on the desirability of having an orthodontist among its membership. Dr. Cline is an orthodontist practicing in Vancouver.

MOVED by Dr. Brown and SECONDED by Dr. Ham

THAT Dr. Bagnall be nominated to serve a further term on the Committee.

MOVED by Dr. Husband and SECONDED by Dr. Copp

THAT nominations for a replacement for Dr. Bagnall be closed.

CARRIED.

MOVED by Dr. Copp and SECONDED by Dr. Burton

THAT Dr. Pagé be nominated to serve a further term on the Committee.

MOVED by Dr. Beveridge and SECONDED by Dr. Aldous

THAT nominations for a replacement for Dr. Pagé be closed.

CARRIED.

The SECRETARY was instructed to request approval of Council of the election of Dr. Cline, Dr. Bagnall and Dr. Page for three year terms to expire in March 1958.

The CHAIRMAN drew the attention of the meeting to the fact that the Executive of the Committee should be elected annually. The present Executive consisted of the Chairman, the Secretary and Brig. Wansbrough as ex officio members, and Dr. Racey and Dr. Ham.

DR. COPP said that the members of the Executive were to be congratulated on the fine work which they had done during the past year, a statement in which the rest of the Committee heartily concurred.

DR. HAM suggested that the members of the Executive rotate. DR. MURRAY felt that if this were done care should be taken that all members of the Executive were not changed at one time; he also pointed out the advisability of having the members of the Executive relatively closely situated geographically in order to avoid possible delay in dealing with urgent matters which might arise.

MOVED by Dr. Copp and SECONDED by Dr. Beveridge

THAT the present Executive be re-appointed for the coming year.

MOVED by Dr. Burton and SECONDED by Dr. Williams

THAT nominations to the Executive be closed.

CARRIED.

The present Executive was therefore re-elected to office for the coming year.

12. Publications by Grantees

The SECRETARY reported that no reprints of papers by grantees had been received since the 20th Meeting.

13. Distribution of "Bibliography on Caries Research"

The SECRETARY reported that 5 additional copies of Dr. Bagnall's "Bibliography on Caries Research" had been sold since the 20th Meeting. A total of 275 copies have now been sold or otherwise distributed, leaving a supply of 42 copies on hand at the present time.

14. Grantees' Reports, and Periodicity of Meetings.

The CHAIRMAN suggested that the Committee consider the advisability of asking grantees to submit reports on their projects annually instead of twice a year. He felt that this would not only save grantees considerable time, but would also result in better reports.

It was the general feeling of the Committee that if reports were requested only for the Spring meeting of the Committee, it would be possible to get a clearer picture of the work sponsored by the Committee at a more advanced stage of completion. DR. McDOUGALL pointed out that projects are not very far under way by the time first reports have normally been requested for the Fall meetings.

The CHAIRMAN said that if only one report a year were required of grantees, it would hardly be necessary for the Committee to meet twice a year in view of the fact that the Fall meeting - a short one - is usually devoted almost entirely to the consideration of grantees' reports.

MOVED by Dr. Lussier, and SECONDED by Dr. Aldous

THAT grantees be asked for an annual report only, and that the Committee hold one meeting a year.

CARRIED.

It was AGREED that the annual meeting of the Committee be held in the Spring, so that applications for grants and reports by grantees could be considered at the same time.

In connection with the reports by grantees, DR. COPP stated that he was strongly in favour of the Committee's decision to request only a single annual report; he also felt that the reports should be improved considerably.

It was agreed that grantees should be asked to describe their work in much the same way as they would present it in a paper for publication, with outlines of the aims of the research, the apparatus and method used and the results, giving full bibliographic documentation. It was felt that this would be of great assistance to the Committee in making a proper evaluation of the work being done.

DR. COPP also suggested that, at the discretion of the Executive, reports by grantees be sent to outside referees qualified to give an opinion on the research undertaken. It was AGREED that this suggestion be adopted.

15. Location of Future Meetings

The CHAIRMAN reported that some of the members of the Committee had expressed the opinion that the meetings should be held at various centres in rotation, instead of in Ottawa each time. It was felt that this might serve to stimulate interest in the field of dental research. The CHAIRMAN pointed out, however, that since the suggestion had been made, the Committee had received more applications for grants in aid of research than it had money to support and suggested therefore that the question might no longer be suitable for discussion.

DR. BURTON said that by holding meetings in Toronto, for example, less expense would probably be involved since many of the grantees were located there and would be readily available for discussion with the Committee without the necessity of spending funds on their attendance at meetings in Ottawa.

In reply to DR. COPP's question as to the practicability of carrying the necessary records and files to meetings outside of Ottawa, the SECRETARY said that with the present scale of operation this would not constitute a special problem.

After some further discussion, it was MOVED by Dr. Beveridge and SECONDED by Dr. Pagé.

THAT until there are good and valid reasons for moving, the Committee continue to hold its meetings in Ottawa.

CARRIED.

16. Appreciation of Services

The CHAIRMAN, speaking on behalf of the entire Committee, thanked the retiring member, Dr. R.H. McDougall for his valuable service to the Committee, and expressed the general feeling that although existing regulations

made his immediate re-appointment impossible it was hoped that he might become a member of the Committee again at a later date.

17. Date of Next Meeting

Establishment of the date for the next meeting of the Committee was left in abeyance, pending information as to the time in which other Council Committees would meet in the Spring of 1956. A suitable date will be set by the Chairman and the Secretary.

The meeting adjourned at 2:35 p.m.

SUMMARY OF WORK

Three papers have issued out of the research work of 1953-54 in spite of the great disturbances encountered along with the trans-location of this Department to its permanent quarters:

1. Electrophoretic and Autoradiographic Detection of a Sulfomucoprotein Synthesized by the Gastric Gland of the Rat
Marc Grevier (by invitation) and Leonard F. Belanger.

Metabolic interrelations between gastric intrinsic hemopoietic factor and vitamin B₁₂ have been demonstrated. This intrinsic factor was shown to be a mucoprotein originating from the stomach wall. On the other hand, radioisotopes have been localized in the mucous neck cells of the fundic gland and in the mucous cells of the pyloric and cardiac glands.

Fractions from gastric mucus of young rats previously treated with H-B₁₂ were submitted to electrophoresis. The electrophoretograms were stained routinely. Contact autoradiographs were also obtained from them and compared with the previous. The mucoprotein fraction was the only one to demonstrate radioactivity. The electrophoretic properties of the sulfomucoprotein were found to be identical to those of the mucoprotein previously isolated from human gastric juice and possessing anti-anemic properties.

It seems thus highly evident that the intrinsic factor mucoprotein is a sulfomucoprotein, and that its site of synthesis is the mucous neck cells of the fundic gland and the mucous cells of the pyloric and cardiac glands.

(Presented at the Canadian Physiological Society meeting of October, 1954. In press with Comptes Rendus de la Société de Biologie, Paris.)

APPENDIX A

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Mechanism of bone and tooth formation in the light of autoradiography and histochemistry.

Grantee: Dr. L.-F. Bélanger,
University of Ottawa.

Project No.: DR-52 Amount of Grant: \$3,470.00

SUMMARY OF WORK

Three papers have issued out of the research work of 1953-54 in spite of the great disturbances encountered along with the trans-location of this Department to its permanent quarters:

1. Electrophoretic and Autoradiographic Detection of a Sulfomucoprotein Synthesized by the Gastric Gland of the Rat.
Marc Crevier (by invitation) and Leonard-F. Bélanger.

Metabolic interrelations between gastric intrinsic hemopoietic factor and vitamin B₁₂ have been demonstrated. This intrinsic factor was shown to be a mucoprotein originating from the stomach wall. On the other hand, radiosulphur has been localized in the mucous neck cells of the fundic gland and in the mucous cells of the pyloric and cardiac glands.

Fractions from gastric mucosa of young rats previously treated with H₂S³⁵O₄ were submitted to electrophoresis. The electrophorograms were stained routinely. Contact autoradiographs were also obtained from them and compared with the previous. The mucoprotein fraction was the only one to demonstrate radioactivity. The electrophoretic properties of the sulfomucoprotein were found to be identical to those of the mucoprotein previously isolated from human gastric juice and possessing anti-anemic properties.

It seems thus highly evident that the intrinsic factor mucoprotein is a sulfomucoprotein, and that its site of synthesis is the mucous neck cells of the fundic gland and the mucous cells of the pyloric and cardiac glands.

(Presented at the Canadian Physiological Society meeting of October, 1954. In press with Comptes Rendus de la Société de Biologie, Paris.)

2. Autoradiographic Visualization of Ca^{45} Intake by Normal and Pathological Cartilage "in Vitro". L.-F. Bélanger.

Summary. Demineralized sections of normal and pathological cartilage from various sources, were soaked in a weak solution of $\text{Ca}^{45}\text{Cl}_2$ and coated with fluid photographic emulsion. The autoradiographic record has shown an apparently specific pattern of Ca^{45} intake. Comparisons of these images and those obtained with S^{35}O_4 in vivo and also the negativating effect of hyaluronidase seems to indicate a relationship between Ca^{45} intake and the relative local distribution of sulfated mucopolysaccharides in the tissue.

(In print with the Proceedings of the Society for Experimental Biology and Medicine. Presented at the meeting of the Histochemical Society, 1954.)

3. Autoradiographic Visualization of the Entry and Transit of S^{35} Methionine and Cystine in Bones, Teeth and Various Other Tissues of the Growing Rat. Leonard-F. Bélanger.

Summary. Bio-synthetized S^{35} methionine and cystine were introduced subcutaneously into 8 day old rats subsequently sacrificed at intervals of 1 hr. to 4 weeks. The fate of the radioactive material retained after formaldehyde-ethanol fixation, was mapped via integrated autoradiographs. In both instances, the maximal concentration of S^{35} was found at 24 hrs. in the keratogenous zone of the hair follicles. The zones of growth in bone and dentine demonstrated considerable S^{35} synthesis with subsequent "migration" of the tagged portions. The cartilage heads of long bones showed a large intake by the hypertrophic cells followed by secretion into the matrix and turnover. The newly formed enamel was highly radioactive after S^{35} methionine but not after S^{35} cystine. In both instances, the general keratinized surfaces, dry and wet, demonstrated radioactivity.

Other tissues which synthetized both S^{35} amino acids were in order of intensity of the autoradiograph, the pancreatic acini, the mucosal glands of the intestine and stomach, the liver, the cortex of the kidney, the spleen, particularly the white pulp, the hemopoietic marrow and the central nervous system, especially the grey matter. Progressive migration along the villi with time, revealed the pattern and rate of growth of the intestinal mucosa.

(In preparation for the Anatomical Record and also for presentation at the Sixth International Congress of Anatomy, Paris.)

APPENDIX B

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Bacteriology of periodontal disease. I. Experimental fusospirochetel infections with mixtures of pure cultures.

Grantee: Dr. J. B. Macdonald,
University of Toronto.

Project No.: DR-35 Amount of Grant: \$2,300.

SUMMARY OF WORK

The objective in the studies reported has been the development of methods for studying quantitatively the bacterial content of exudate from experimental guinea pig mixed infections. Media have been developed for cultivating the organisms which result in the appearance of distinctive colonies which can be readily identified. Methods are described for obtaining estimates of the viable count along with appropriate statistical controls. Although the methods appear to be satisfactory for 2 of the 4 strains concerned, they are not satisfactory for the other 2. The character of the counts of one of the latter suggest the existence of a mechanism in the exudate exerting a controlling influence on its growth.

1. Strain K80 forms a film on heart infusion blood agar surfaces rather than distinctive colonies.
2. Colonies of strain K92 are not clearly distinguishable from colonies of J83 in mixed anaerobic culture.
3. Growth of strain K110 requires a growth factor elaborated by strain J83. The growth on agar surfaces would depend on the presence of J83 or some other source of the growth factor.

A number of media and methods were tried to overcome these difficulties.

It was found possible to overcome the spreading of strain K80 and to produce distinctive colonies by plating on a medium prepared from Difco thioglycollate broth with 3 per cent agar and 10 per cent sheep blood. The colonies were smooth, opaque, mottled grey, slightly heaped, and iridescent, about 1 mm. in diameter. Strain J83 produced very distinctive colonies on the same medium both aerobically and anaerobically. They were less

PROGRESS REPORT

The purpose of the studies reported here has been the development of methods to study the in vivo numerical relationships of the 4 essential components of a mixed infection. Satisfactory methods would make it possible to answer the following questions:

1. Is there any uniformity or pattern to the total viable count from exudates?
2. Is there a difference in total count or in distribution of the 4 organisms in localized as opposed to spreading lesions?
3. Do the 4 organisms tend to establish a stable relationship to each other in terms of proportions?
4. Does any one of the strains consistently predominate in the exudate?

I. Development of satisfactory media.

The standard laboratory media and methods used in these studies and reported previously are unsatisfactory for counting studies for the following reasons:

1. Strain K80 forms a film on heart infusion blood agar surfaces rather than distinctive colonies.
2. Colonies of strain K92 are not clearly distinguishable from colonies of JR3 in mixed anaerobic culture.
3. Growth of strain K110 requires a growth factor elaborated by strain JR3. The growth on agar surfaces would depend on the presence of JR3 or some other source of the growth factor.

A number of media and methods were tried to overcome these difficulties.

It was found possible to overcome the spreading of strain K80 and to produce distinctive colonies by plating on a medium prepared from Difco thioglycollate broth with 3 per cent agar and 10 per cent sheep blood. The colonies were smooth, opaque, mottled grey, slightly heaped, and iridescent, about 1 mm. in diameter. Strain JR3 produced very distinctive colonies on the same medium both aerobically and anaerobically. They were less

than 1 mm. dry, opaque yellow and very rough. Strain K92 produced large distinctive colonies on heart infusion medium with 3 per cent agar and 10 per cent sheep blood. These colonies were about 1 mm. or more in diameter, smooth, grey-white with a flat semi-translucent margin and a central opaque white papilla.

Strain K110 presented more difficulties. A number of media were tried in which was used a filtrate of Micrococcus aureus in peptone water. (This filtrate contains the growth factor.) The filtrate in some instances was incorporated in the medium, in others it was spread or sprayed on the surface of the medium. The filtrate was also used as diluent for the exudate when preparing serial dilutions for plating. None of these methods proved satisfactory. Growth was erratic and the colonies were generally minute and not well pigmented. To obtain satisfactory growth on a surface it was found necessary to grow strain K110 in association with an organism which produces the growth factor. Two methods employing a culture of Micrococcus aureus were employed. One was to streak a heart infusion plate with Micrococcus aureus along two parallel lines about 1" apart, and to permit a drop of diluted exudate to run between them by tipping the plate. Diffusion of the growth factor permitted good growth of strain K110. Equally satisfactory and more economical, was a method whereby exudate dilutions were dropped directly on top of drops of a culture of Micrococcus aureus.

II. The counting method.

Counts were made from colonies growing in drops of diluted exudate placed on the surface of appropriate media. The diluent was nutrient broth containing 20 mg. per cent colloidal graphite which served to disclose the outline of counting drops made from the dilutions. Tests indicated that the presence of the graphite did not influence the resultant counts. Graphite was found to be more satisfactory than calcium oxalate which has been recommended for the same purpose because the graphite remained in suspension whereas the calcium oxalate tended to precipitate, carrying organisms down with it.

When aqueous drops are delivered to agar surfaces they frequently fail to wet the surface immediately and as a result they skid across the plate like a drop of mercury. This was prevented by depositing finely divided calcium oxalate on the plates (in the form of a spray, 0.2 per cent suspension) 2 hours before use and then drying in the incubator as recommended by Rosebury et al (1954).

Serial tenfold dilutions of exudate aspirated from guinea pig lesions were prepared in the nutrient broth plus graphite.

Dilutions extended from 10^{-1} to 10^{-9} . Experience indicated the countable dilution ranges for each organism as follows:

JR3	- 10^{-2} to 10^{-4}
K80	- 10^{-2} to 10^{-4}
K110	- 10^{-3} to 10^{-8}
K92	- 10^{-7} to 10^{-9}

Drops of the indicated dilutions were delivered on the appropriate media from a 22 gauge needle by means of a tuberculin syringe. (Needles chosen delivered 100 drops /1 ml.) Sixteen drops of each dilution were placed on each of 5 plates making a total of 80 drops at each dilution. The same plates served to count both strains JR3 and K80 (Difco thioglycollate 3 per cent agar 10 per cent blood). Separate sets of heart infusion blood plates were used for strain K92 and for strain K110.

In the case of strain K110 the method was as follows: A 24 hour culture of Micrococcus aureus in peptone water plus 20 mg. per cent colloidal graphite was delivered as drops onto heart infusion blood agar - 16 drops per plate. When the drops had dried the exudate dilutions were dropped directly on top of the Micrococcus aureus drops.

All cultures were incubated anaerobically for 14 days. Counts were made under a stereoscopic microscope at a magnification of 10 x. Results were recorded for each countable dilution on a special form (Fig. 1).

III. Analysis of data.

Analysis was made for homogeneity of counts between plates by determining chi-square values based on the average drop-count on each ($\bar{x}_d$) compared to the average drop count for all 5 plates ($\bar{x}_D$). This provided a statistical control of the method.

Counts of this type should follow a Poisson distribution (Eisenhart and Wilson, 1943). A second analysis was made to determine the goodness of fit of the distribution to a Poisson model with the same average drop count. The chi-square method was again employed as shown on Fig. 1. Where counts follow a Poisson distribution the variance is equal to the mean and hence the standard error of the mean (S_m) is equal to $\sqrt{\text{mean}}$ or $\sqrt{G.T.}$ the grand total for the dilution, and the best estimate of the mean colony count. When the data satisfactorily fit the Poisson distribution the best estimate of the true count per ml. of undiluted exudate could be obtained by this formula:

$$\left[G.T. \quad 1.96 \quad \sqrt{G.T.} \right] \times \frac{100}{\text{total no. of drops}} \times \text{dilution factor.}$$

RESULTS

Data were obtained from 8 different exudates. In general the method proved satisfactory for strains JR3 and K80. Counts followed a Poisson distribution and the tests for plate homogeneity showed satisfactory uniformity between plates. Estimates obtained from different dilutions were comparable (e.g. Table I) and there were no significant differences in counts of JR3 uncubated aerobically or anaerobically. Counts of JR3 and K80 varied about one thousand fold from different exudates. Both JR3 and K80 counts were comparatively low. JR3 counts (per ml.) ranged from 2.8×10^4 to 1.8×10^7 and K80 counts ranged from 2.0×10^5 to 1.8×10^8 .

The method was less satisfactory for strain K110. There was variability between plates to an extent which made most of the data unusable. Nevertheless sufficient data were obtained to suggest that the counts tended to conform to the Poisson distribution. It seems probable that the variability indicated a lack of uniformity in the cultural conditions - possibly related to the distribution of the growth factor.

Analyses for plate homogeneity and conformity to Poisson distribution indicated that the counts of strain K92 were satisfactory. However a curious phenomenon was observed regularly in counting colonies of this organism. The actual counts (not estimates) regularly appeared to be roughly equivalent in consecutive tenfold dilutions in the countable range. This meant that estimates derived from such counts would differ from each other by a factor of 10. This relationship is illustrated in Table 2. The explanation for this behaviour is not known. It might be associated with clumping of this organism or possibly the presence of some inhibitor in the exudate which is gradually diluted out. Whatever the explanation it is clear that the colony counts for this organism do not represent an estimate of the numbers of organisms in the exudate.

DISCUSSION

The important features of this counting method are as follows:

1. The choice of media makes the different strains easily distinguishable.
2. The use of plates sprayed with calcium oxalate permits accurate placement of the drops.
3. The use of colloidal graphite accurately discloses the position and outline of every drop.

4. The above 2 features permit the use of 16 drops on a plate with a great saving in media. A total of 80 counts at each dilution greatly increases the accuracy of the method without waste of media.
5. The use of 80 drop counts provides enough data to test goodness of fit to the Poisson model at each dilution.
6. The test of plate homogeneity provides good statistical control of the method.

CONCLUSION

Although progress has been made toward developing suitable methods for quantitating the organisms in exudates, the methods are not yet satisfactory. Progress in this field beyond the present point will require the development of satisfactory quantitative methods for both in vitro and in vivo investigations.

Table 1.

Average drop counts of strain JR3 in exudate #647

Plate	Dilution		
	10^{-4}	10^{-5}	10^{-6}
1	17.38	1.86	.25
2	18.24	1.63	.31
3	18.50	2.19	.25
4	17.88	1.94	.56
5	19.00	1.69	.56
Average	18.34	1.86	.39

Table 2.

Average drop counts of consecutive tenfold dilutions of strain K92

Dilution	Exudate		
	#654	#638	#633
10^{-7}		3.3	13.9
10^{-8}		3.4	2.2
10^{-9}	39.3	3.2	11.4
10^{-10}	38.0		
10^{-11}	30.7		

C. P. #	EXUDATE TYPE	ORGANISM	DATE	BK.	P.											
PLATE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1																
2																
3																
4																
5																
TOTAL DROPS (TD) =																
$\chi^2 = \frac{15 \sum (\bar{x}_D - \bar{\bar{x}}_D)^2}{\bar{\bar{x}}_D}$																
G.T. =																
$S_M = \sqrt{GT} =$																
DILN. CONF. LIMITS = 1.96 $S_M =$																
RANGE = TO																
COUNTS/DROP OBSERVED																
POISSON ($\bar{x}_D =$)																
DIF																
DIF ²																
(O - E) ²																
E																
FOR POISSON FIT $\chi^2 =$																
ORGANISM PER ML EXUDATE = G.T. x $\frac{100}{TD}$																
D.F. =																
P =																
DILN. FACTOR =																
$S_M = \sqrt{GT} \times \text{DILN. FACTOR} \times \frac{100}{TD}$																

APPENDIX C

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: The cultivation and characteristics of Bacteroides nigrescens.

Grantee: Dr. J. B. Macdonald,
University of Toronto.

Project No.: DR-50 Amount of Grant: \$400.00

SUMMARY OF WORK

The studies reported relate to the growth factor required for certain strains of Bacteroides nigrescens.

Indol production by Bacteroides nigrescens could not be demonstrated before visible growth.

Harvesting and suspending organisms from blood agar for use in a tube assay technique appeared to destroy most of the cells.

Filtrate of staphylococcus growth transferred to assay cylinders or filter paper strips on blood agar failed to support growth.

Concentration of the volume of the active filtrate at 0°C. or 60°C. by distillation yielded a product which would not support growth.

The growth factor was found to be dialyzable.

Cylinder assay.

Heavily seeded blood agar plates were used. Glass cylinders (10 mm. i.d.) were placed on the plates and 0.5 ml. of filtrate of staphylococcus growth in peptone water added to each cylinder. After 10 days anaerobic incubation no growth was found on the plates in the area surrounding the cylinders. Controls streaked with staphylococcus showed good growth and growth even occurred surrounding a contaminant on one of the cylinder-plates. The results suggested that the concentration of growth factor was inadequate to support growth or that the growth factor was inactivated on the agar surface (e.g. by oxidation).

Progress Report

The studies reported relate to the growth factor required for growth of certain strains of Bacteroides nigrescens.

Production of indol.

Strain K110 produces indol. Cultures in Difco thioglycollate broth plus a filtrate of staphylococcus growth in peptone water were tested daily following inoculation to determine the earliest point at which indol formation could be demonstrated. It was found that indol formation could not be demonstrated before visible growth appeared.

Tube assay.

Further work was done on the possibility of using the micro-nutritional assay of Shills and Horowitz (1953) dependent on growth of the test organism in seeded agar tubes overlaid with the growth factor. Earlier attempts to use this method failed because of non-viability of the washed cells used for seeding the agar tubes. To circumvent this problem unwashed cells were tried. These were scraped from blood agar surfaces, suspended in thio-glycollate broth (without agar) plus Tween 80 (1:30,000). The suspension was emulsified in a tissue grinder to disperse the growth. The resultant heavy turbid suspension was inoculated into melted Difco thioglycollate 0.6 per cent agar medium, using 1 ml. of suspension to 15 ml. of melted medium. The suspension was also plated on blood agar and cross-streaked with staphylococcus as a control. The seeded medium was transferred to 1 mm. tubing. After solidification 0.1 ml. of a filtrate of staphylococcus growth in peptone water was layered on top. After 10 days anaerobic incubation the tubes showed growth of 3 or 4 isolated colonies only. The control also showed very light growth. It was concluded that the manipulation involved in preparing the suspension had rendered most of the cells nonviable.

Cylinder assay.

Heavily seeded blood agar plates were used. Glass cylinders (10 mm. i.d.) were placed on the plates and 0.5 ml. of filtrate of staphylococcus growth in peptone water added to each cylinder. After 10 days anaerobic incubation no growth was found on the plates in the area surrounding the cylinders. Controls cross-streaked with staphylococcus showed good growth and growth even occurred surrounding a contaminant on one of the cylinder-plates. The results suggested that the concentration of growth factor was inadequate to support growth or that the growth factor was inactivated on the agar surface (e.g. by oxidation).

Filter paper strips.

Strips of sterile filter paper soaked in filtrate of staphylococcus growth in peptone water were laid across blood agar plates seeded with strain K110. No growth occurred in the zone adjacent to the strips although a control cross-streaked with staphylococci showed good growth. This result again suggested that the concentration of growth factor was too low to initiate growth or that the growth factor was inactivated.

Concentration of growth factor.

Efforts were made to concentrate the growth factor in peptone water by reducing the volume to one tenth. This was done by a vacuum pump at 60°C. and also at approximately 0°C. The procedure was completed in both cases in 1 hour. The concentrated material did not support growth of strain K110.

Dialysis.

Peptone water (40 ml.) in dialysis tubing was inoculated with staphylococci. The tubing was suspended in 100 ml. of Difco thioglycollate broth and incubated for 24 hours. The dialysate was then tubed and inoculated with strain K110. Controls consisted of strain K110 in ordinary Difco thioglycollate broth (negative control) and strain K110 in thioglycollate broth plus filtrate of staphylococcus growth in peptone water (positive control). Growth was found after 10 days incubation in the dialysate and in the positive control but not in the negative control. The growth factor appears to be dialyzable.

Cylinder assay.

Heavily seeded blood agar plates were used. Glass cylinders (10 mm. i.d.) were placed on the plates and 0.5 ml. of filtrate of staphylococcus growth in peptone water added to each cylinder. After 10 days anaerobic incubation no growth was found on the plates in the area surrounding the cylinders. Controls cross-streaked with staphylococcus showed good growth and growth even occurred surrounding a contaminant on one of the cylinder-plates. The results suggested that the concentration of growth factor was inadequate to support growth or that the growth factor was inactivated on the agar surface (e.g. by oxidation).

APPENDIX D

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Continuing studies in electromyographic techniques.

Grantee: Dr. H. Jenkins,
University of Toronto.

Project No.: DR-37

Amount of Grant: \$225.00

SUMMARY OF WORK

The sample for this study originated in 1953 with a group of 40 school children aged $5\frac{1}{2}$ - $7\frac{1}{2}$ and evenly distributed as to sex. The sample this year consists of 34 of the original individuals consisting of 19 boys and 15 girls with an age range of $6\frac{1}{2}$ - $8\frac{1}{2}$ years.

Method.

Records are available for 37 of the original sample (3 sets are not being used) for two years at approximately a year apart. These records consist of (1) models (2) electromyographic tracings of the temporal muscle (3) cephalometric radiographs.

(1) Model assessment

Models were assessed for any change in the classical picture of occlusal relationships for deciduous molar to cuspid dentition. Change from neutroccclusion to Class II Division I relationship was noted in five individuals.

Models have also been assessed for the presence of permanent molars erupting in neutroccclusion and end to end.

(2) Electromyographic records were classified as to

- (a) activity or
- (b) non activity of the post-temporalis muscle in both physiological rest position and contact. In addition those individuals exhibiting activity are being further classified on the basis of whether this activity was bilateral or unilateral.

Several questions may be answered:

- (1) are the muscle patterns consistent with each other for two years? - Yes.
- (2) were the occlusal changes noted previously on models associated with changes in muscle activity? - No.
- (3) was the shift to Class II Division I in five individuals associated with activity of the post-temporalis muscle? - Yes.

(3) Cephalometrics

Project I

Cephalometric radiographs of this year's sample are being surveyed in a cross-sectional study to establish normal standards for face dimensions. The basic aim is to divide the various anatomical structures of the face into their component parts and to project the dimensions of these parts into two planes of space. This permits analysis area by area to determine the location of the deficiency if present and its dimensional orientation. We are fortunate that some of the samples have now deviated as this will permit us to make comparisons with our normal standards.

Project II

A cross-sectional study of asymmetry of normal individuals employing antero-posterior films.

Standards of asymmetry of orthodontically normal Canadian children in two planes of space employing four points in the maxillo-facial complex and one point in the mandible are being developed.

To date all records have been gathered, cephalometric tracings made and the measurements and electromyographic data noted above are being tabulated for statistical analysis. Interim reports have been presented to the Toronto branch of the International Association of Dental Research.

APPENDIX E

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Chemical mechanisms of dental caries.

Grantee: Dr. J. J. Rae, (C.T. Clegg)
University of Toronto.

Project No.: DR-1

Amount of Grant: \$ 1,080.

SUMMARY OF WORK

Since our last annual report (March 1954) two papers have been submitted for publication to the Journal of Dental Research and these are included as Sections A and B of this report. Section C is a paper on Lactic Acid Production by Saliva which has also been submitted to the Journal of Dental Research. Section D is a progress report on the Bactericidal Material towards l.b.a. prepared from tooth enamel.

The following statements summarize the results.

1. Glucose inhibits ammonia production even in solutions buffered at pH 7.0 showing that the inhibition is not altogether attributable to a lowering of pH. The stimulating effect of glucose on ammonia production in the presence of urea is not apparent when buffered solutions (pH 7.0) are used.

2. Concentrations of glucose as low as 0.1% (no urea) inhibited ammonia production by saliva. Urea concentration below 0.3% (no glucose) did not cause a rise in pH on incubation of the saliva. High concentrations of urea (10%) inhibited ammonia production. The ratio of glucose to urea for optimum stimulation of ammonia production is approximately 1:1.

3. Lactic acid production and l.b.a. counts of saliva are unrelated. Zero-count salivas produce average amounts of lactic acid. The addition of urea seems to enhance slightly lactic acid production by saliva-glucose mixture.

4. Additional information on the bactericidal substance has been accumulated but attempts to identify this material have not yet been crowned with success.

PROGRESS REPORT

A. Ammonia Production in Saliva*

J.J. Rae, Ph.D., H.M. Shemilt, B.A. and C.T. Clegg, Phm.B.,
Chemistry Department, University of Toronto, Toronto.

In a previous paper (1) we reported on the presence of an ammonia producing mechanism in saliva which, on incubation of the saliva for 24 hours at 37° produced five to ten times the amount of ammonia originally present. The addition of glucose (2.5% final concentration) to the incubating mixture inhibited ammonia production. The addition of urea (1.5% final concentration) enhanced ammonia production showing the presence of an active urease in saliva. The addition of both glucose and urea stimulated the production of ammonia beyond the amount produced when urea alone was added.

In a second paper (2) it was shown that there was no relationship between the activity of the ammonia-producing mechanism and caries activity as measured by lactobacillus counts. The urease activity of the salivas studied also showed no relationship to caries activity. The stimulating effect of glucose on ammonia production in the presence of urea was observed in all the salivas studied.

In an attempt to shed further light on the mechanism of glucose inhibition of ammonia production on the one hand and glucose stimulation of ammonia production in the presence of urea on the other, the following experiments were performed.

Experimental (1) The relationship between pH and ammonia production in saliva is shown in the following table. The Folin-Bell (3) method was used to determine ammonia. A veronal-acetate buffer was used to control the pH.

Table I
The Relationship Between pH and Ammonia Production in Saliva

Tube No.	pH		Ammonia Content mg%
	At start	At 24 hrs.	
1	4.42	4.60	10.9
2	5.50	5.79	14.3
3	6.62	6.81	31.4
4	7.98	7.92	22.5
5	8.46	8.42	9.5

These results indicate that the optimum pH range for ammonia production is 6.5 - 7.0.

* This research was assisted by a grant from the Associate Committee on Dental Research, National Research Council, Ottawa.

(2) The inhibitory effect of glucose on ammonia production might be due to the fact that the added glucose forms a substrate for acid-producing bacteria which would produce acid and thus lower the pH below the optimum for ammonia production. To test this theory a series of buffered tubes (pH 7.0) were incubated with and without glucose. The results obtained are shown in Table II.

Table II
The Effect of Glucose on Ammonia Production by Saliva Buffered at 7.0.

Tube No.	Glucose ml.20%	Saliva ml.	pH after 18 hrs	NH ₃ Content mg%
1	1	2	6.70	none
2	0	2	7.00	16.0
3	1	2	6.75	trace
4	0	2	7.00	17.5

Since glucose inhibited ammonia production even when the tubes were buffered at pH 7.0 the lowering of the pH is not the only factor in the inhibition of ammonia production by glucose. The buffer used in these experiments was found to have a slight inhibitory effect on ammonia production which accounts for the somewhat lower ammonia values in this experiment.

The stimulating effect of glucose on ammonia production by saliva-urea mixtures could be simply a pH effect. The acid production by bacterial enzymes acting on the glucose could keep the pH at the optimum for ammonia production (7.0) for a longer time and so more ammonia would be produced.

To shed light on this question, the ammonia production with and without added glucose by buffered urea-saliva solutions was observed. The results are shown in Table III.

Table III
The Effect of Glucose on Ammonia Production by Urea-Saliva Mixtures Buffered at pH 7.0.

Tube No.	Saliva ml.	Urea ml.20%	Glucose ml.20%	pH after 18hrs.	NH ₃ Content after 18hrs. mg%
1	2	1	1	7.20	92.5
2	2	1	0	7.70	85.0
3	2	1	1	7.25	92.5
4	2	1	0	7.75	87.0

These results indicate that the stimulating effect of glucose could be attributed to acid keeping the pH near the optimum for ammonia production. Glucose in buffered solutions showed no stimulating effect on ammonia production.

Discussion and Conclusions. A certain amount of disagreement seems to exist in the literature as to whether ammonia in saliva is related to dental caries. Saliva contains bacterial enzyme systems capable of producing acid which could decalcify enamel and other bacterial enzyme systems capable of producing ammonia. Our work has shown that the addition of glucose to saliva inhibits ammonia production and that this inhibition is not altogether attributable to the acid produced lowering the pH beyond the limit for ammonia production. Glucose per se in buffered solutions inhibits ammonia production.

Glucose when added to urea-saliva mixtures enhances ammonia production and in this case pH seems to be decisive factor. In buffered solutions (pH 7.0) the stimulating effect of glucose on ammonia production is no longer observed.

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1. Ballantyne, R.M., Clegg, C.T., Rae, J.J. and Lawford, F.A. J.D. Res., 30: 385, 1951.
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3. Folin, O. and Bell, R.D. J. Biol. Chem., 29: 329, 317.

(Paper submitted to J. D. Res., May 13, 1954.)

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B. Ammonia Production in Saliva^X

C.T. Clegg, Phm.B., and J.J. Rae, Ph.D., Chemistry Department, University of Toronto, Toronto, Canada.

Previous work (1,2) has shown that there is an ammonia producing mechanism in saliva which is inhibited by glucose. This glucose inhibition was still exhibited when solutions buffered at pH 7.0 were used showing that the inhibition was not altogether due to a lowering of the pH.

A urease is also present in saliva which produces large amounts of ammonia when urea is added. If glucose is added to these urea-glucose mixtures the ammonia production is greater than when urea alone is used.

★ This research was assisted by a grant from the Associate Committee on Dental Research, National Research Council, Ottawa.

When the pH is maintained at pH 7.0 the stimulating effect of glucose on ammonia production in the presence of urea is no longer observed. This would indicate that pH is the decisive factor and that glucose acts to maintain the pH near the optimum for ammonia production and does not exert any stimulating effect by itself.

Experimental. Wide variations in the final concentration of glucose (without added urea) from 0.1 to 10% produce comparable drops in pH on incubation with saliva. The concentration of glucose does not seem to be related to the drop in pH at these levels.

Wide variations in the final concentration of urea (without added glucose) from 0.1 to 5% produce comparable amounts of ammonia and the pH rose from 7.5 to 9.0 in all tubes. However 10% urea inhibited ammonia production.

The effect of varying the ratio of glucose to urea on ammonia production was investigated and the results are shown in Table III.

Table III
The Effect of Variations in the Ratio of Glucose to Urea on Ammonia Production in Saliva

Tube No.	Concentration		Ratio Glucose/Urea	Final pH	NH ₃ mg%
	Glucose	Urea			
	%	%			
1	4.0	0.2	1:0.05	5.6	235
2	2.0	0.2	1:0.1	5.4	270
3	5.0	1	1:0.2	8.6	277
4	1.0	1	1:1	8.8	283
5	1.0	3	1:3	8.3	245
6	1.0	5	1:5	8.7	70
7	2.0	-	-	4.0	none
8	-	1.0	-	9.0	190

These results show that when the urea concentration falls below 1/10 of the glucose it is not sufficient to prevent a drop in pH even though the ammonia production stays at a fairly high level. Excessive amounts of urea in the presence of glucose inhibited ammonia production.

The amounts of glucose and urea used in most of the earlier experiments (2% glucose and 1.5% urea) resulted fortuitously in a ratio of glucose to urea which gave the optimum stimulation of ammonia production by glucose.

Summary. In an attempt to shed further light on the ammonia-producing mechanism in saliva experiments were performed in which the concentrations, when used separately, and the ratios, when used in conjunction, of urea and glucose added to the saliva were varied. Only small amounts (0.1%) of glucose (no added urea) were needed to obtain large drops in pH. Concentrations of urea (no added glucose) below 0.3% did not cause a rise in pH on incubation. High concentrations of urea (10%) inhibited ammonia production.

A ratio of glucose to urea of about 1:1 exhibit optimum stimulation of ammonia production. If the urea is lowered below 0.2% (added glucose) the drop in pH is not prevented. If the urea is raised above 3% inhibition in ammonia production is observed.

References

1. Ballantyne, R.M., Clegg, C.T., Rae, J.J. and Lawford, F.H. J. Dent. Res., 30: 385, 1951.
2. Rae, J.J., Shemilt, H.M., Clegg, C.T. Unpublished results.

(Paper submitted to the Journal of Dental Research, December 3, 1954.)

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C. Lactic Acid Production By Saliva

J.J. Rae and C.T. Clegg, Chemistry Department, University of Toronto, Toronto, Canada.

One of the fundamental premises upon which many current theories as to the mechanisms involved in dental caries is based is the production of lactic acid by bacterial action on carbohydrate food residues in the mouth, which causes acid decalcification of the enamel (1,2). Since the production of acid is usually accompanied by a drop in pH, measurements of pH have been widely used to determine acid production (3). Such measurements are open to the obvious difficulties inherent in well-buffered solutions and do not indicate the amount of acid present. In an attempt to overcome these difficulties others have measured lactic acid production (4) and have used this as a measure of caries susceptibility. In an attempt to assess the validity of such methods the following experiments were performed.

Experimental. The method used to determine lactic acid was the one described by Barker and Summerson (5) as modified by R.L. Markus (6).

To see how much lactic acid would be produced by zero-count salivas six such salivas were incubated with buffer and 1% glucose. The mixtures contained 1 ml. saliva, 4 ml. buffer and 1 ml. 10% glucose and water to 10 ml. and were incubated for 24 hours at 37°. The results are shown in Table I.

Table I

Lactic Acid Production of Zero-Count l.b.a. Salivas

Tube No.	Lactic Acid Produced after 24 hours mg %
1	45.7
2	18.7
3	14.3
4	16.5
5	41.2
6	44.2

When salivas with variable l.b.a. counts were used the results obtained are shown in Table II.

Table II

Relation Between Lactic Acid Production and l.b.a. Count of Salivas

Tube No.	l.b.a. Count	Lactic Acid mg %
1	21000	16.7
2	30000	61.0
3	60000	20.6
4	200000	19.1
5	300000	45.0

These few results indicate that there is no relation between l.b.a. count of salivas and the mg % lactic acid produced on incubation. The fact that zero-count salivas produced considerable and variable amounts of lactic acid would indicate that lactic acid production cannot be used as a reliable measurement of l.b.a. concentration. The obvious implication of the results with zero-count salivas is that there are present in the saliva acid-producing bacteria other than lactobacillus acidophilus.

The pH of saliva when incubated at 37° rises from pH 7.0 to 8.0 if no glucose is added. If glucose is added the pH drops and even quite low concentrations of glucose are suffic-

ient to cause this drop. Wide variations in glucose concentrations above the minimum seem to have little or no effect on the drop in pH or on the amount of lactic acid produced. The total volume in each tube was 10 ml. and 2 ml. of saliva was used in each case.

Table III
Relation Between Glucose Concentration, pH and Lactic Acid Production

Tube No.	Glucose Conc.	pH after 18 hours	Lactic Acid mg % after 18 hours
1	0	8.0	0
2	0.1	4.2	39.4
3	0.5	4.6	31.2
4	1.0	4.5	31.8
5	5.0	4.6	40.0
6	10.0	4.5	31.8

The optimum pH for lactic acid production in the presence of 1% glucose was found to be 6.5.

When urea was added to saliva-glucose mixtures the pH does not drop probably due to the fact that the urea in the saliva produces ammonia which neutralizes the acid formed. Strangely enough the lactic acid production seems to be stimulated somewhat by the addition of urea. Possibly the ammonia produced keeps the pH near the optimum for lactic acid production (6.5) for a longer time and thus more lactic acid is produced.

Table IV
Effect of Adding Urea to Glucose-Saliva Mixtures on Lactic Acid Production and pH

Tube No.	Saliva	Glucose ml. 10%	Urea ml. 20%	pH at end	Lactic Acid mg %
1	2	-	1	9.0	none
2	2	-	1	9.0	none
3	2	2	-	3.5	10.0
4	2	2	1	8.8	15.0
5	2	2	1	8.6	12.7

Summary. Lactic acid production by saliva seems to be a poor criterion for lactobacillus concentration since zero count salivas produce considerable amounts of lactic acid and in the few cases studied there is no apparent relation between lactobacillus count and lactic acid production.

Quite low concentrations of glucose (0.1%) are sufficient for lactic acid production. The optimum pH for lactic acid production is 6.5. The addition of urea to saliva-glucose mixtures, even though it keeps the pH of the incubation mixture above 7.0, does not inhibit lactic acid production and in this experiment even enhanced somewhat the lactic acid production.

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(Paper submitted to Journal of Dental Research, January 15, 1955.)

D. A Bactericidal Substance Towards Lactobacillus Acidophilus Prepared From Tooth Enamel.

Ground tooth enamel (60 mesh) prepared by the Manly-Hodge technique, which involves a density separation by means of bromoform, was found to inhibit the growth of l.b.a. Powdered calcium phosphate which resembles tooth enamel chemically did not show such a bactericidal action under similar conditions. Bromoform (0.05 ml.) added to the culture broth did not inhibit growth.

The following procedure for the preparation of this inhibiting substance has been evolved. Powdered tooth enamel is shaken with either bromoform or a bromine (10%) dissolved in chloroform solution. The solids separated by filtration and dried at 160° for six hours. The boiling point of bromoform is 150°. The powder is then extracted with methanol or other suitable solvent producing what is called Filtrate I. A subsequent treatment of the tooth enamel with bromine in chloroform produces a further crop of the inhibiting substance.

Numerous procedures and tests have been applied to Filtrate I and to the brown gummy residue produced by evaporating aliquots of Filtrate I to dryness but so far no pure substance has been separated or identified.

Qualitative tests indicate the presence of halogen probably chlorine. No sulphur, nitrogen, phosphorus or bromine was found to be present. A micro test for the presence of bromoform gave a negative test.

By means of solubility tests the substance probably belongs to hydrocarbons or their halide derivatives.

Ashed powdered tooth enamel when treated with bromoform did not produce the inhibiting substance.

Paper chromatography procedures as yet have produced no separation of the substance into fractions.

The inhibiting substance acts at very low concentrations 0.1 ml. of Filtrate I (0.42 mg. solids) inhibited growth of l.b.a. in 4 ml. of culture broth.

The inhibiting substance when added to saliva was found to prevent acid production in the presence of glucose as well as inhibiting ammonia production.

APPENDIX F

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Development of a rapid method for grinding thin sections of plastic-embedded teeth.

Grantee: Dr. B. N. Kropp,
Queen's University.

Project No.: DR-54

Amount of Grant: \$650.

SUMMARY OF WORK

The construction and operation of the machine designed for cutting thin sections of plastic-embedded calcified teeth and bone is described. The procedure is given for cutting single sections, and also for cutting serial sections.

Although designed for use with the method developed for grinding and polishing thin sections of teeth and bone, the machine may be used for cutting plastic-embedded materials quite independently of the grinding and polishing procedure.

Fixed to the outer face of the shaft casing is the guard, made of $\frac{1}{2}$ " sheet brass, and forming a circular disk, 4" diameter, with two flat sides. The inner guard plate (10, fig. 1, 8) is attached to the shaft casing. The guard has a front opening 1 $\frac{1}{2}$ " long where the saw rest is designed to be cut, and a circular opening at the back (3, fig. 1, 8) 1 $\frac{1}{2}$ " diameter, to which is attached a 1" length of $\frac{1}{2}$ " brass tubing which serves as a vacuum connection. The outer plate of the guard (3, fig. 4) has a 1 $\frac{1}{2}$ " center hole to permit clearance for the box cut on the shaft. This plate is also cut away, as shown in figs. 4 and 5, to permit clearance of the specimen past the saw. The plate is held in place by three 1" h. bolts, $\frac{3}{8}$ " x $\frac{1}{2}$ ", (seen in figs. 4, 5, 7) which are held in threaded holes in the inner guard plate (10, figs. 1, 8).

(2) Specimen Holding and Fixing Assembly. This is mounted on a hardwood block (9, fig. 8 seen also in figs. 5, 7), 2 $\frac{1}{2}$ " high, 6 $\frac{1}{2}$ " wide, 7 $\frac{1}{2}$ " front to back. Just inside the two outer corners holes are bored for $\frac{5}{16}$ " bolts, $\frac{1}{2}$ " long, (9, fig. 8, seen also in figs. 6, 7) which pass through the flange holes in the plywood panel and are held by threaded metal strips on the under side of the panel. Two bolts at the inside corners of the hardwood block (10, fig. 8, also

PROGRESS REPORT

The Apparatus

The apparatus consists essentially of two parts: (1) shaft and saw assembly, and (2) the assembly for holding the work and feeding it into the saws.

(1) Shaft and saw assembly. The $\frac{1}{2}$ " shaft (T, fig. 8) is 12" long and mounted by means of two vertical supports on a 5" x 9" base (U, fig. 8). This base plate is firmly bolted to a $\frac{3}{4}$ " plywood panel, and to the latter is also bolted the work-feeding assembly described below. The shaft casing (S, fig. 8) has an outside diameter of $1\frac{3}{8}$ ". Grease plugs (D, figs. 1, 8) are placed near each end and lubricate the shaft bearings. At one end of the shaft is mounted a flat-surface pulley wheel series designed to give motor speed (1725 rpm) 2100 and 2600 rpm. The opposite end of the shaft (A, fig. 1) is threaded for a distance of $\frac{3}{4}$ ". Permanently mounted on the shaft so as to provide a flat surface stop for the first saw, is an aluminum collar, 1" diameter (B, figs. 1, 8). The mounted saws are locked firmly in place on the shaft by means of an aluminum washer (I, fig. 3) and a $\frac{7}{8}$ " hex nut (H, figs. 3, 8). When more than one saw is mounted on the shaft, an aluminum spacer 1 mm. thick and $1\frac{1}{2}$ " diameter is placed between adjacent saws.

Fixed to the outer face of the shaft casing is the guard, made of $\frac{3}{8}$ " sheet brass, and forming a circular box, 4" diameter, with two flat sides. The inner guard plate (C, figs. 1, 8) is attached to the shaft casing. The guard has a front opening $1\frac{1}{4}$ " long where the saws meet the specimen to be cut, and a circular opening at the back (G, fig. 3), about $1\frac{1}{8}$ " diameter, to which is attached a 2" length of $1\frac{1}{8}$ " brass tubing which serves as a vacuum connection. The outer plate of the guard (J, fig. 4) has a $1\frac{1}{2}$ " center hole to permit clearance for the hex nut on the shaft. This plate is also cut away, as shown in figs. 4 and 5, to permit clearance of the specimen past the saws. The plate is held in place by three r. h. bolts, $\frac{1}{8}$ " x $1\frac{5}{8}$ ", (seen in figs. 4, 5, 7) which are held in threaded holes in the inner guard plate (C, figs. 1, 8).

(2) Specimen Holding and Moving Assembly. This is mounted on a hardwood block (V, fig. 8 seen also in figs. 5, 6), $2\frac{1}{2}$ " high, $6\frac{1}{2}$ " wide, $7\frac{1}{2}$ " front to back. Just inside the two outer corners holes are bored for $5/16$ " bolts, $2\frac{1}{2}$ " long, (Q, fig. 8, seen also in figs. 6, 7) which pass through similar holes in the plywood panel and are held by threaded metal strips on the under side of the panel. Two bolts at the inside corners of the hardwood block (W, fig. 8, also

figs. 6, 7) pass through slots in the inner face of the block. Bolted to the block (four $\frac{1}{4}$ " bolts) is a plate of $\frac{3}{8}$ " brass, $4\frac{1}{4}$ " x $4\frac{3}{4}$ " (Z, fig. 8). The bearing surface guides (Y, fig. 8) are of $\frac{3}{8}$ " brass, $\frac{3}{4}$ " x $4\frac{1}{4}$ ". A movable plate is operated by a $\frac{3}{8}$ " screw (R, fig. 8, also fig. 6) to give movement in a front to back axis. The top of this movable plate is also provided with a pair of fixed bearing guides which are mounted at right angles to the lower pair of guides as shown in fig. 8. A section of aluminum tubing, (M, fig. 8, also figs. 5, 6, 7), $1\frac{1}{4}$ " I.D., 2" O.D., 2" long, is mounted on a brass plate and is capable of moving laterally from right to left. It is moved by a micrometer head (O, fig. 8, also figs. 5, 7). On the underside of the plate holding the micrometer is a pair of springs (P, fig. 8) fixed at one end to the underside of the plate and at the other to the supporting piece of the micrometer head. The springs provide assurance of lateral movement of the top plate.

Operation

Specimens to be cut are first embedded in transparent plastic by a method previously published (Kropp, Stain Technology, vol. 29, March, 1954) and further developed (DR-54, Report of February 5, 1954^{*}). Essentially the method consists of thoroughly drying the specimen of bone or tooth, embedding in plastic with the aid of a metallurgical press so as to form a disc or cylinder 1" in diameter. During this process one must have in mind that the disc or cylinder will be cut transversely and the specimen oriented accordingly. Specimen size, must of course be within the limits of cylinder size, but it is advantageous to reduce the specimen size whenever possible so as to provide the maximum amount of supporting plastic. Although the diameter of the disc or cylinder cannot be varied the length can be varied by altering the amount of plastic used in forming the cylinder.

After removal from the press the embedded specimen is attached to the flat surface of a Plexiglas rod having a diameter of $1\frac{1}{4}$ " and a length of 6", by means of a few drops of methylene dichloride which permanently fuses the two plastic surfaces. The wing nut N, fig. 8 (also figs. 6, 7) is removed. The rod with the attached specimen is inserted into the aluminum holder with the specimen end opposite the saws, and the wing nuts L and X, fig 8 (also see figs. 6, 7) tightened. Wing nut N is now replaced but not tightened. The work is now

^{*} See Proceedings of the 19th Meeting of the Associate Committee on Dental Research, Appendix "M".

moved in a plane parallel to the shaft by means of a calibrated micrometer head. The latter abutts against the movable platform to which the aluminum tube is fixed (see cut-away, fig. 8). The two springs (P) prevent lateral play in this member. When the correct position of the specimen with reference to the saws in this plane has been obtained, wing nut N is tightened.

The motor and vacuum are now turned on. The effect of the latter is two-fold. 1) It carries off dust and debris resulting from the cutting operation, and 2) the stream of air drawn over the specimen and saws is a very effective coolant. The vacuum apparatus we use is a small domestic-type vacuum cleaner with cylindrical body which may be mounted in any position and location convenient to the apparatus. It is recommended, however, that when in continuous operation, the open end of the vacuum cylinder should be fitted with a hose connection leading to a fume cupboard or an open window. The odor produced in cutting bone and teeth is sometimes offensive. It is advisable also to reduce the length of the hose connection between cutting machine and vacuum to a minimum.

Cutting

The saws are so-called "jeweller's saws", made of high speed steel, $3\frac{1}{2}$ " O.D., 0.014" thick, $\frac{1}{2}$ " center hole, and 120 teeth (Circular Tool Co. Inc., Providence, R.I.). These saws have a slight clearance - a critical factor in the operation of the machine. The clearance, though slight, is sufficient to permit the cut sections to pass between adjacent saws with little contact between specimen and saws. Without saw clearance the work will "jam" when more than one saw is used, as in multiple cutting.

To cut single sections a single saw is mounted on the shaft and the specimen oriented in the plane parallel to the shaft, and in front of the saw, as described above. With the motor and vacuum on, the knob (R, fig. 8) is slowly turned clockwise until the saw begins to cut into the plastic. The rate with which the specimen is thus fed into the saws must be carefully gauged but is quickly determined by experience. Our method is to turn the knob slowly clockwise until the saw just engages the plastic and then turn the knob slightly counter-clockwise. This is repeated but the specimen is always advanced a little more than it is retracted on each turn. When the saw encounters the specimen within the plastic the forward movement of the work must be watched carefully to avoid forcing. But unless the specimen is unusually hard

or brittle there is no difficulty. Any difficulty that does arise is taken care of by reducing the rate at which the specimen is fed into the saws. If the plastic appears to be melting as it is cut then the speed of the saw is too great or the specimen is being cut too rapidly. The cut is not made completely through the plastic disc, but stops about $\frac{1}{8}$ " short. If the single section is all that is required, the Plexiglas rod and specimen are removed from the machine and the thin disc quickly severed from the rod by a few strokes of a thin brass-back hand saw. If more than one section is needed, the knob is rapidly rotated counter-clockwise so as to withdraw the work from the saw, the calibrated micrometer is then turned the necessary amount so as to cut the next section the required thickness. This is repeated for as many sections as may be required. When completed the specimen is removed from the machine and each section removed in order with the aid of the brass-back saw as described above.

When it is desired to cut serial (multiple) sections of the specimen with a single operation, the procedure is only slightly different. Mount the necessary number of saws on the shaft having in mind the length of the specimen to be cut, and having in mind also that the thickness of the saws (0.014") represents wastage. (It is apparently possible to obtain saws as thin as 0.012" or even 0.010", but we have not yet determined whether their use is feasible.) The specimen is now fed into the saws as in the cutting of single sections. Again the cutting operation is stopped short of completion when there is about $\frac{1}{8}$ " of plastic remaining. The work is removed by the machine and the discs (with the cut specimens) sawed off in order as before. In serial section cutting the minimum thickness of specimen thus far obtained has been 1 mm. (the thickness of the aluminum separator between saws). With single section cutting operations, however, it has been possible to cut specimens of teeth and bone as thin as 0.5 mm. After cutting, the specimen may be ground and polished to the required thinness by the method previously mentioned (Kropp, 1954). After cutting, the plastic surrounding the specimen is usually somewhat cloudy, although this does not interfere with any subsequent grinding or polishing of the specimen. However, it is possible to quickly break the specimen out of the plastic and embed it in fresh transparent plastic if desired.

Fig. 1

View of apparatus stripped down to show shaft, casing, pulleys, motor. A - threaded end of shaft; B - collar on shaft to act as stop for saw; C - inner plate of guard; D - grease plug.

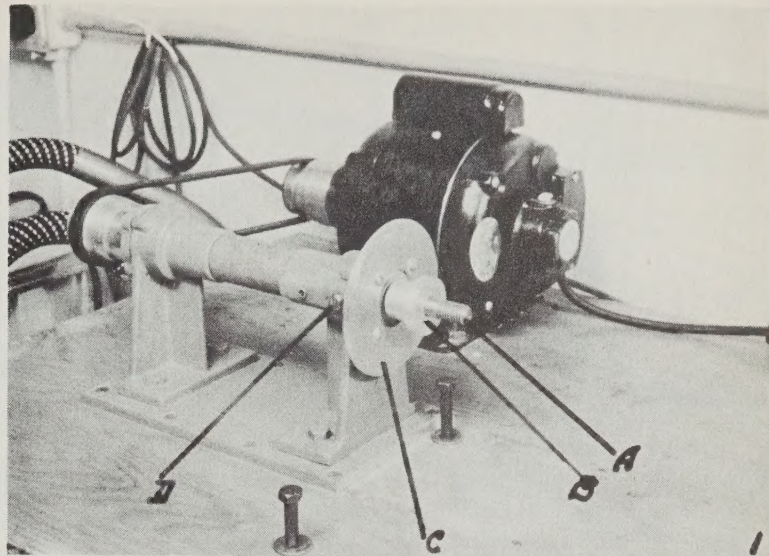


Fig. 2

E - one saw in place on shaft; F - circular section of guard. For cutting single sections this portion of the assembly is completed by inserting the washer (Fig. 3, I), the nut (Fig. 3, H), and the outer guard plate (Fig. 4, J).

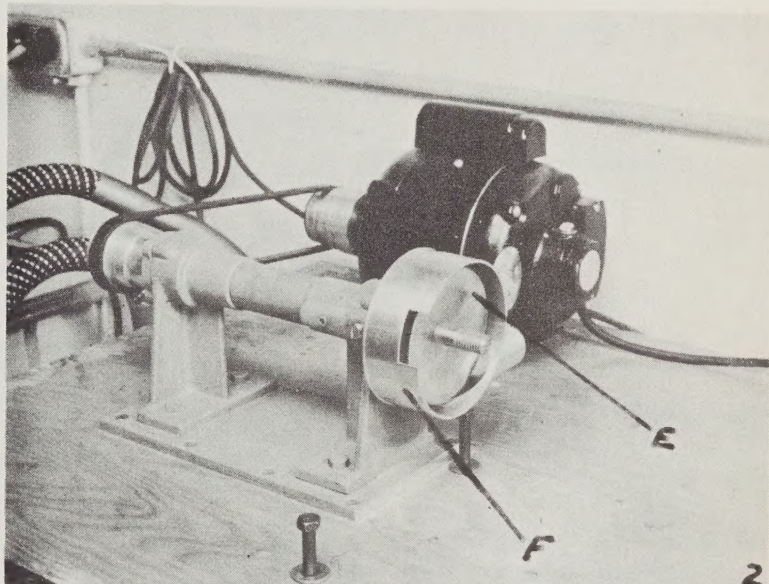


Fig. 3

Gang saws in place (6 in illustration) for cutting simultaneous serial sections. G - vacuum connection; H - hex nut; I - washer.

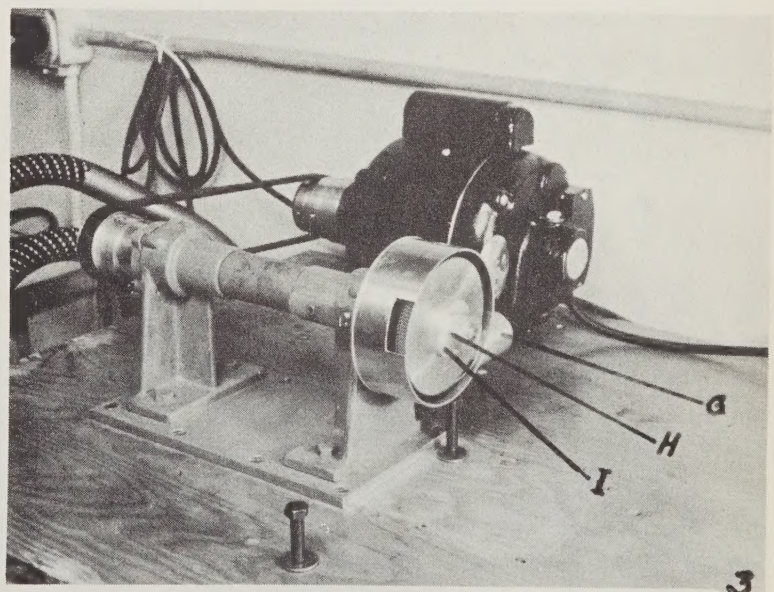


Fig. 4

J - outer guard plate. Completed shaft and saw assembly for multiple cutting.

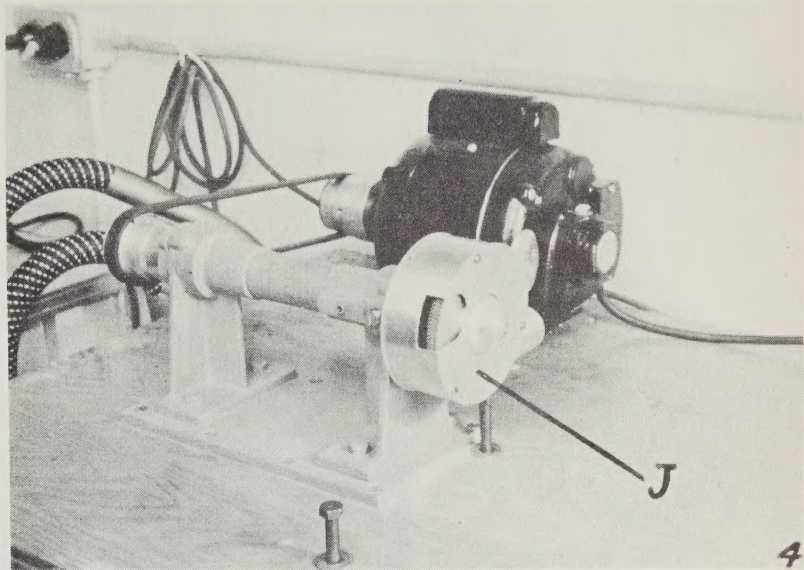


Fig. 5.

Specimen holding and feeding assembly in place.

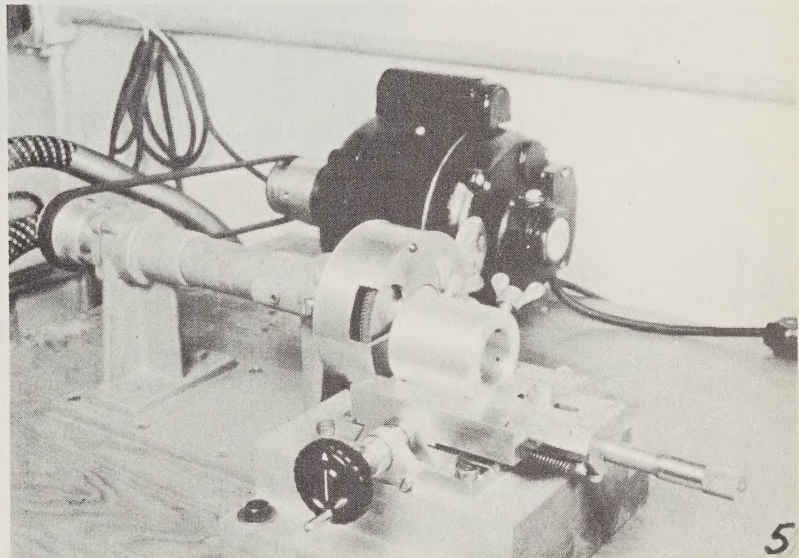
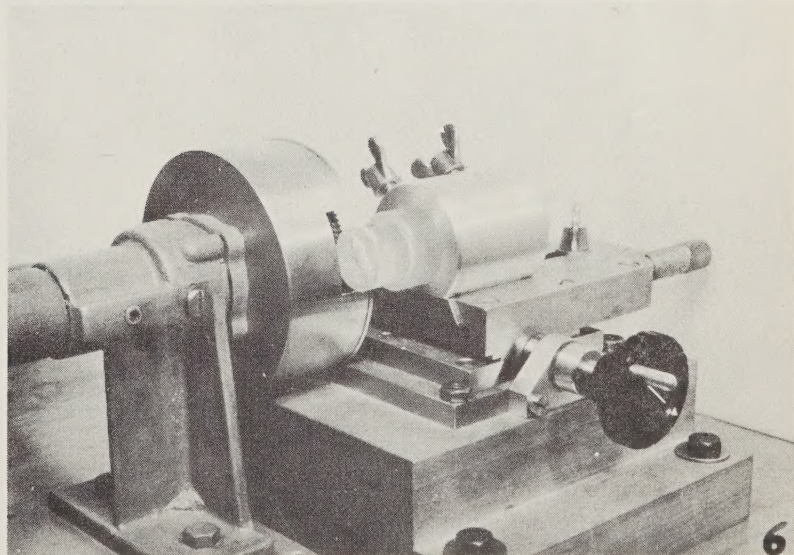


Fig. 6

Plastic embedded specimen in holder and ready for cutting.



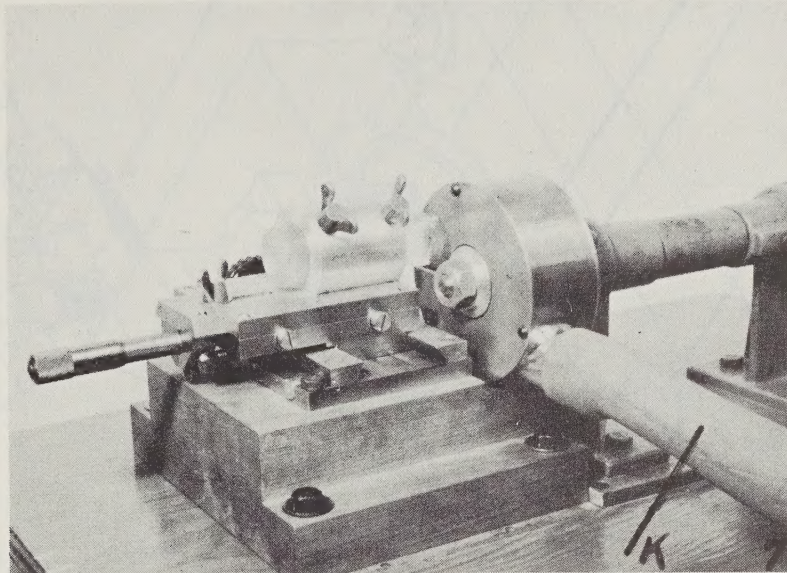
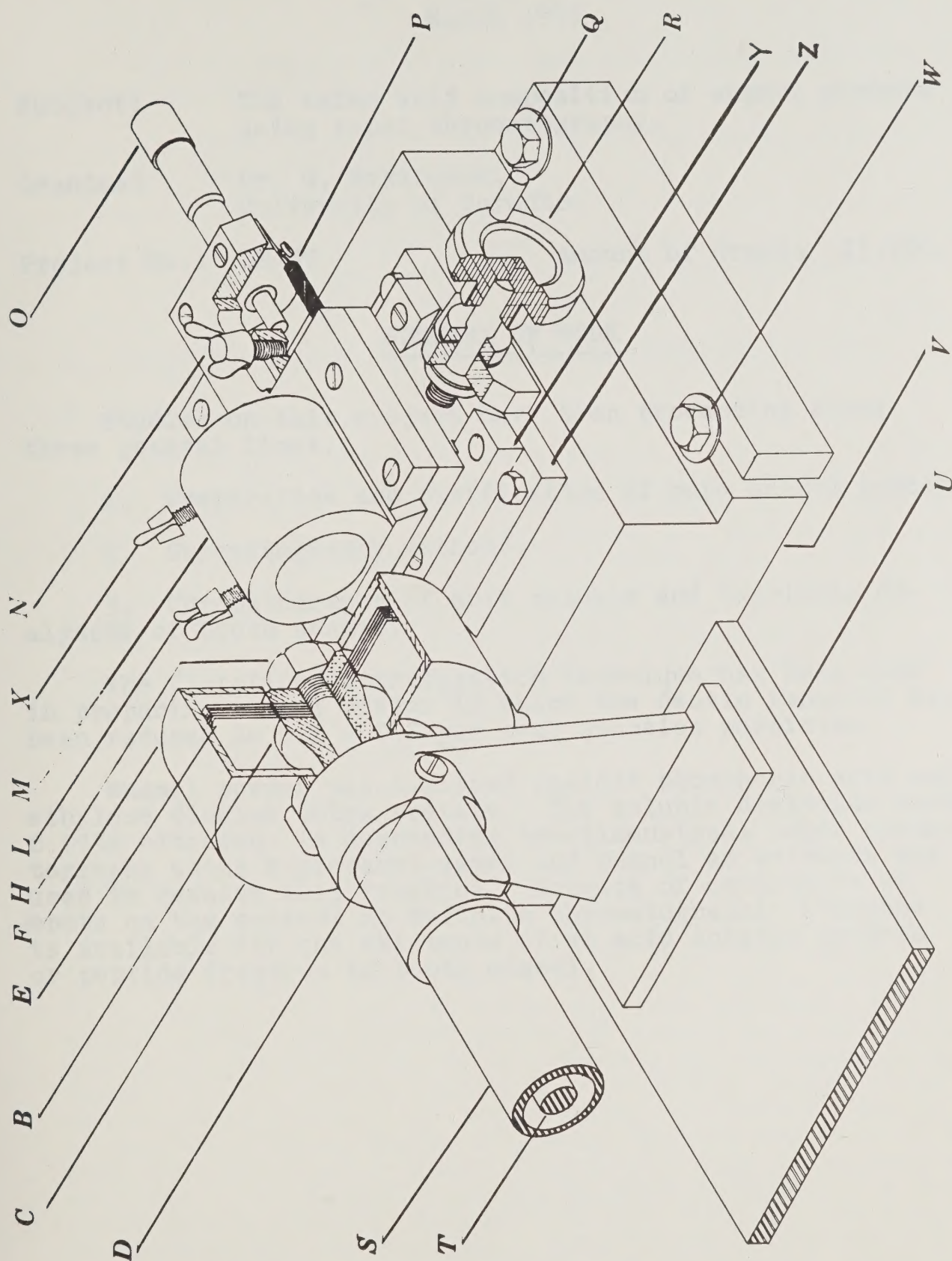


Fig. 7

View of assembly shown in Fig. 6 from read.
K - vacuum hose connection.



Fi. 8

APPENDIX G

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: The amino acid composition of enamel protein using paper chromatography.

Grantee: Dr. G. Nikiforuk,
University of Toronto.

Project No.: DR-57 Amount of Grant: \$3,500.

SUMMARY OF WORK

Studies on this project have been proceeding along three general lines.

1. Preparation and purification of pure enamel powder.
2. Chromatography methods.
3. Chromatography of acid soluble and insoluble dialysate of tooth enamel.

The flotation-centrifugation technique has been used in preparing enamel powder in which the dentin impurity has been reduced to 0.1 - 0.2 per cent junction particles.

Enamel powder was dialyzed against phosphoric acid and ethylene diamine tetra acetate. The soluble dialysate contained 0.059% nitrogen. A descending two-dimensional paper chromatography using N-propanol-water and phenol as solvents was used to resolve this fraction. Because of occurrence of spots on the control no definite chromatographic evidence is available for the existence of an acid soluble protein or peptide fraction in tooth enamel.

PROGRESS REPORT

Work on this project has been proceeding along the following general lines:

1. Preparation and purification of enamel powder.
2. Chromatography methods
 - (a) Washing of filter paper
 - (b) Solvent systems
3. Chromatography of enamel dialysates
 - (a) Chromatography of the soluble fraction of the enamel dialysate.

1. Preparation and Purification of Powdered Enamel

A. Preparation

Sound extracted teeth were stored in acetone and later dried at 100°C. for 6 hours. This permitted enamel to be chipped off readily. The exterior surfaces were lightly ground with a diamond stone to remove all visible pit and fissure material. The cleaned fragments were pulverized in a diamond mortar, after which metal particles were removed magnetically. It was found that the impurity in terms of dentin particles was greatest in the larger particle sizes. The powder that passed a 100-mesh was found most suitable for subsequent purification.

B. Purification of enamel

Two techniques have been tried: (1) Flotation by use of liquid with graduated density. A tall glass cylinder is partly filled with one organic liquid (bromoform) and the remainder of the cylinder filled with acetone -- miscible with the first but lighter. The mixture is mixed gently using a 'spiral' wire stirring rod commencing at the junction of the two liquids and gradually extending along the cylinder length. This gives a mixture in which the density is graduated more or less uniformly from the lower to the higher value. The enamel powder is poured into the cylinder and separates according to the density of the components, enamel (2.89 - 3.00); dentin (2.05 - 2.35). This method was satisfactory for initial separations. However it was difficult to get the desired degree of purity because the heavier enamel particles appeared to carry some dentin impurities during settling. (2) Flotation-centrifugation technique. A bromoform-acetone mixture (density adjusted to 2.75) was used to separate enamel from dentin. Three parts of the mixture to 1 part of enamel powder was used. The powdered enamel was

stirred gently for 2 minutes and then centrifuged. This was repeated from four to eight times until the dentin impurities were reduced to a point where 10 - 20 "junction" particles were observed in a count of 1,000. Enamel particles were differentiated from dentin on the basis of different refractive indices -- dentin (1.56) and enamel (1.60). A liquid with a refractive index of 1.59 prepared by mixing 76 vol. % of α -chloronaphthalene and 18 vol. % of a liquid petrolatum was found most suitable for viewing the powdered enamel. The flotation centrifugation method is used in all subsequent purifications of enamel.

Enamel with impurity of 1 - 2 % "junction particles" was prepared in sufficient quantities to permit preliminary analysis. A junction particle usually comprised 1 part of dentin to approximately 10 parts of enamel. Allowance is made for the dentin impurity during quantitative analysis on the basis of the fact that dentin contains 40 times as much organic matter (3.38 per cent nitrogen) as enamel.

C. Demineralization

A batch of pure enamel powder (weighing 100 mgs.) was placed in a cellophane sac. The sac is agitated in a beaker containing 1.5 litres of 0.5 per cent phosphoric acid or 0.25 M ethylene diamine tetraacetate, pH 7.5. The diffusate is replaced every day for four days or until tests for calcium were negative. Zephiran chloride (.012 % final concentration) is added to prevent growth of organisms. The final diffusate is replaced with distilled water which is changed twice daily for four days. At the end of this time the dialysis sac is presumed to contain only non-diffusible organic molecules.

After completion of this process the insoluble protein is separated from the supernatant fluid by centrifugation (2,500 G's for 2 hours) or by filtering. The supernatant was concentrated from 30 ml. to 0.05 ml. by lyophilization. The insoluble fraction was washed with alcohol and ether, dried and stored at 4°C.

2. Chromatography Techniques

A. Washing of filter paper

Purification of the filter paper (Whatman No. 3) results in marked improvement in the chromatography of amino acids and peptides. The advantages vary but include frequently the formation of more circular spots, cleaner

separation and more rapid 'running'. Washing of the paper involved seven stages and requires 52 days. Large numbers of sheets of paper (60) may be washed at once. The procedure used is essentially as developed by Hanes (1954) and is summarized as follows:

Extraction per		Volume	Duration	Suction
Stage	Block of 60 sheets			
1	2N acetic acid	5 litres	3 days	No suction
2	Water	7 "	4 "	Continuous
3	0.5N lithium hydroxide	12 "	22 "	Intermittent
4	Water	10 "	9 "	"
5	0.1% (w/v) calcium acetate	4 $\frac{1}{2}$ "	3 "	Continuous
6	Water	8 "	5 "	"
7	95% (v/v) ethanol	6 "	4 "	"

The first block of paper is permitted to imbibe the first extractant, a weak acid, for three days. Suction is then applied and a pale yellow effluent drawn from the suction head. The extraction with 0.5 N lithium hydroxide is the most important part of the process. Under the influence of this agent the structure of the paper fibers appears to be opened up and much non-cellulose material comes slowly into solution. The use of calcium acetate adds divalent cations to the paper, a procedure necessary in order to avoid streaking of the amino acid spots. The final wash with ethanol removes a yellow and fluorescent impurity as well as facilitating drying of the paper.

B. Solvent systems employed

A vast number of solvent systems have been suggested for purposes of separation of amino acids by paper chromatography. Preliminary experiments using gelatin hydrolysates were used to test several solvent systems for their resolving powers and general adaptability to the studies and conditions in the laboratory. The solvents selected on the basis of the above criteria and used in this study are n-propanol-water (80-20 v/v) and phenol (saturated with an aqueous solution containing 6.3 per cent sodium citrate and 3.7 per cent sodium dihydrogen phosphate). Descending two-dimensional chromatography was used with a period of at least one hour equilibration before addition of solvent to trough. Figure 1 illustrates a chromatogram of a gelatin hydrolysate. Sixteen well-defined spots are visible.

3. Chromatography of Enamel Dialysate

A. Soluble non-diffusible fraction

Chromatography studies were started on the soluble component of the enamel dialysate (Stack, 1954). A 100 mg. batch of enamel powder and a control were dialyzed in ethylene diamine tetraacetate and phosphoric acid as described. The resulting concentrated dialysate was chromatographed along with similar material that was hydrolyzed for 24 hours at 125°C. in a sealed ampule using a wax bath. Figures 2 and 3 show the developed chromatograms.

Interpretation of the chromatogram is difficult due to the unexpected spots resulting from the control. Experiments using 0.5 per cent phosphoric acid as the decalcifying solution yield seven spots. Hydrolysis of the dialysate produces no definite changes (Figure 2). Dialysate from ethylene diamine tetraacetate shows a pattern similar to the one obtained by phosphate decalcification. Upon hydrolysis an additional spot is observed (Figure 3). Tracings of the chromatograms of the control (a concentrated material obtained from a dialysis sac without enamel powder, that was subjected to treatment identical with the experimental) are shown in Figure 4.

It is possible that impurities in the dialysis sac are responsible for these spots. Experiments are being set up to determine source of these spots.

On the basis of the preliminary results it is not possible to associate the spots on the chromatograms as soluble components of tooth enamel.

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G-6

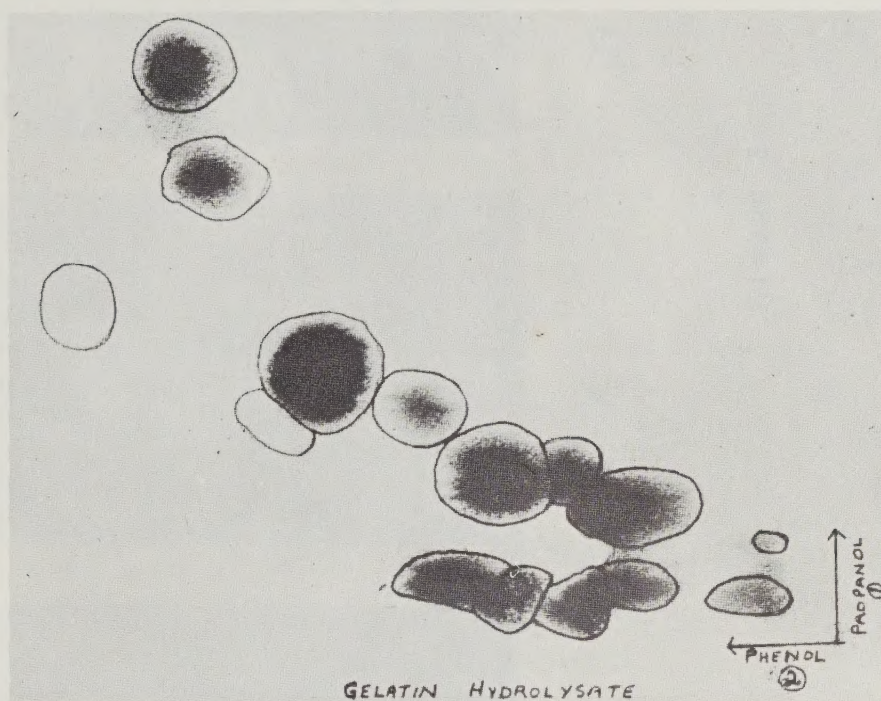


FIGURE 1. RESOLUTION OF GELATIN HYDROLYSATE BY PAPER CHROMATOGRAPHY USING PROPANAL-WATER AND PHENOL AS SOLVENTS.

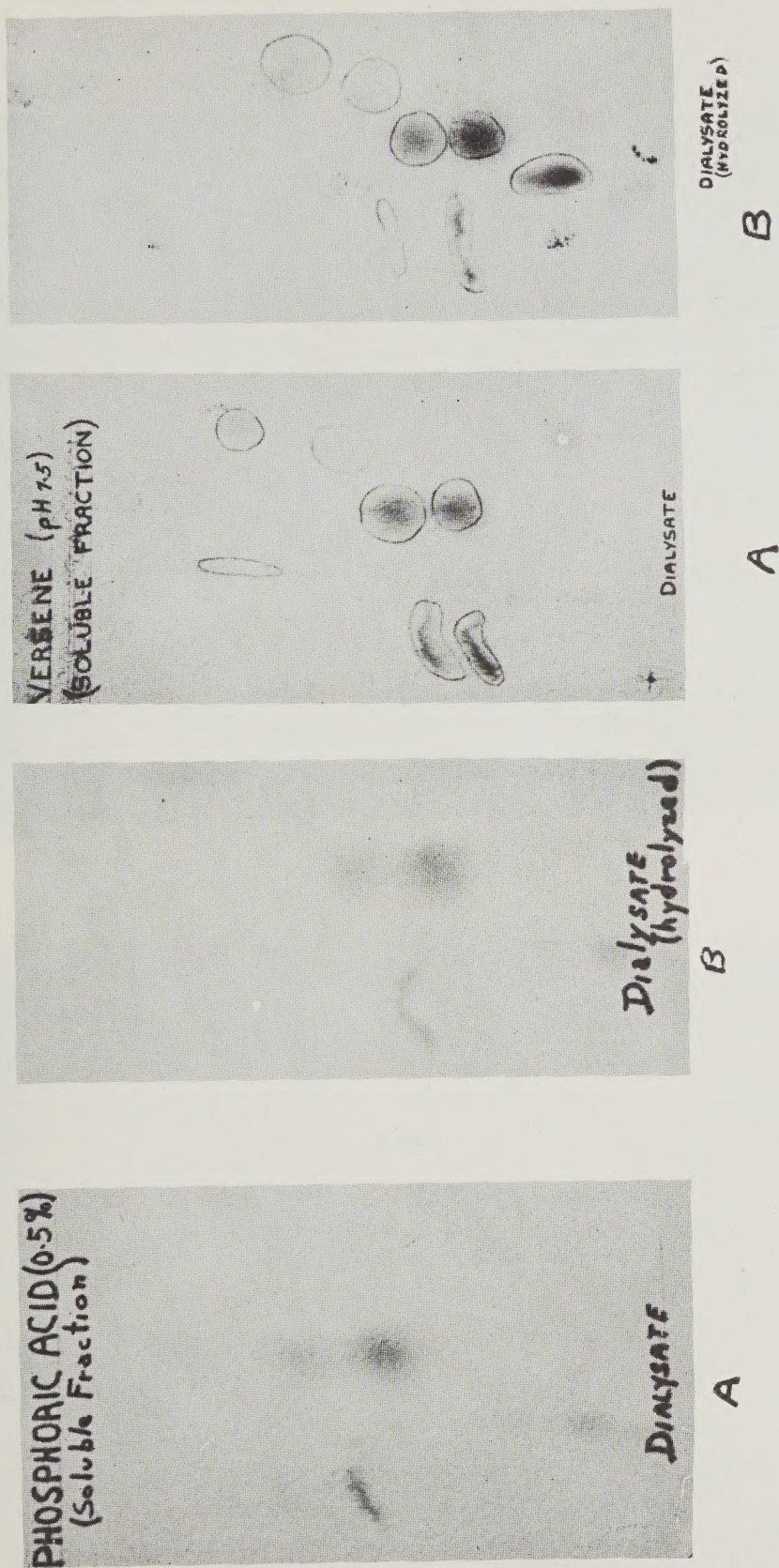


FIGURE 2

FIGURE 3

- A. CHROMATOGRAMS OF DIALYSATE OF ENAMEL POWDER, OBTAINED BY DIALYZING AGAINST PHOSPHORIC ACID (0.5%) AND ETHYLENE DIAMINE TETRA ACETATE (VERSENE) --0.25 M.
- B. CHROMATOGRAMS OF HYDROLYZED FRACTION.

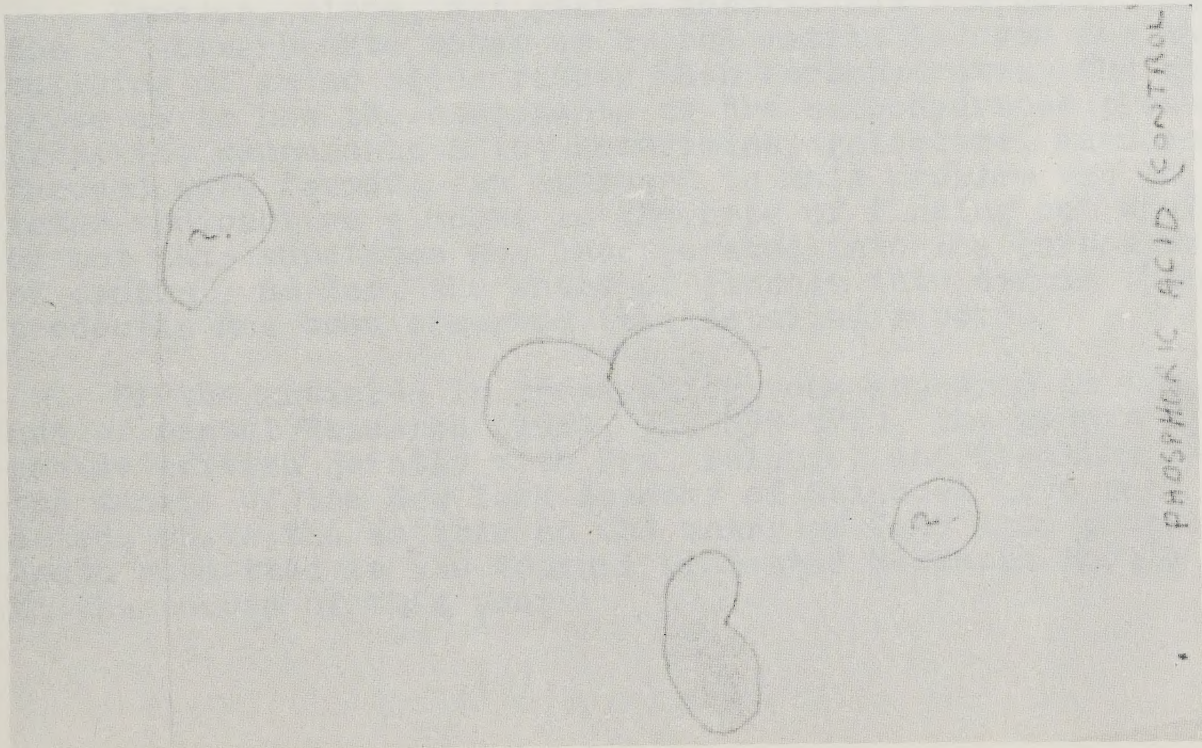
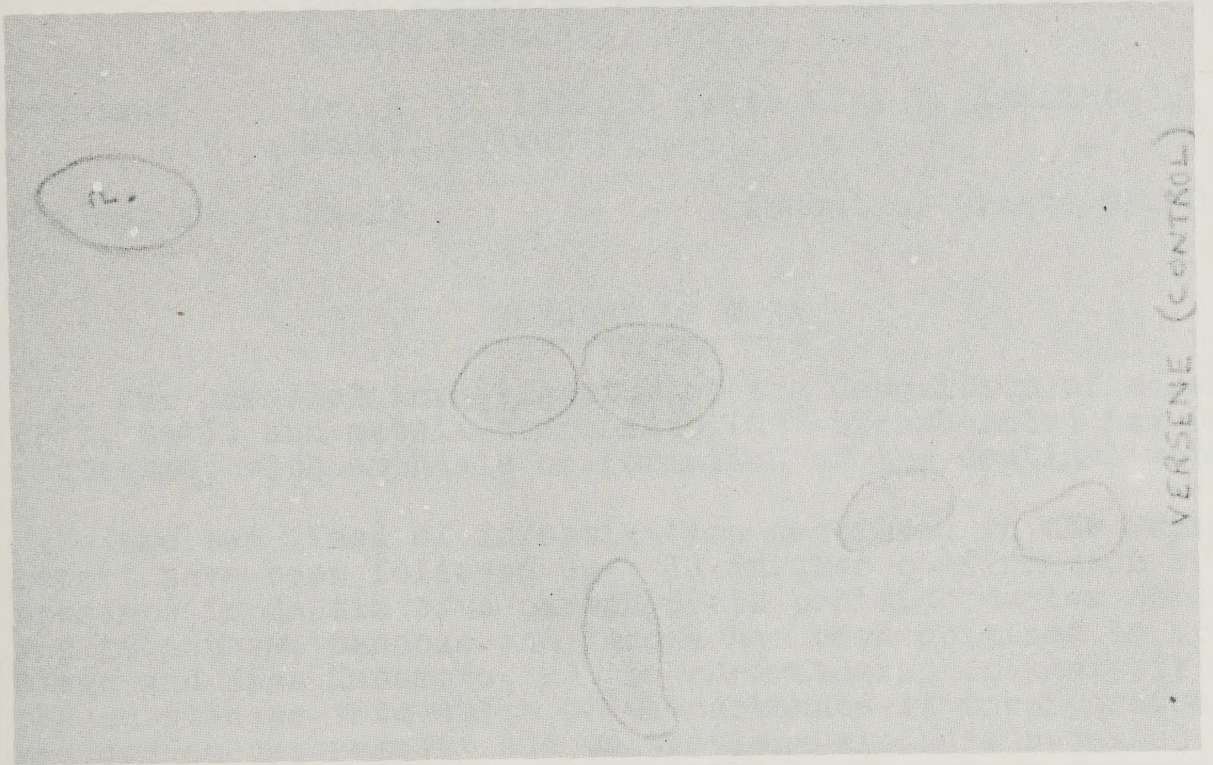


FIGURE 4. TRACINGS OF CHROMATOGRAMS OF CONTROL.

APPENDIX H

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radio-active elements.

Grantee: Dr. C. P. Leblond (with Miss Y. Kumamoto),
McGill University.

Project No.: DR-23

Amount of Grant: \$3,500.00

SUMMARY OF WORK

Our parallel investigation of the deposition of minerals (P^{32} , Ca^{45}) and matrix components (C^{14} -bicarbonate) has been continued, with emphasis on formation of the dentinal matrix.

The two lines of investigation described in the September report have been followed up: 1) further confirmation of the presence in dentin of a carbohydrate protein complex containing galactose, mannose and fucose has been obtained by Dr. Glegg and Miss Kumamoto, 2) the chromatographic analysis of the substances into which C^{14} -bicarbonate is incorporated has yielded no new result, due to the long exposure required to detect the C^{14} -containing material on paper chromatograms by radioautography (4-8 months).

However, since last year's results had indicated that the C^{14} -bicarbonate taken up by the matrix is used for the building of amino acids rather than carbohydrates, the question arose as to how the components of the carbohydrates present (that is, glucuronic acid, hexosamine, galactose, mannose and fucose) were formed. An approach to this problem was to administer radioactive glucose in the hope of finding out whether or not this substance was incorporated into the carbohydrates of dentin. So far, the entry of glucose into dentin through predentin has been observed (see detailed report).

Of the articles in preparation, one appeared in the Journal of Dental Research (1954, 33, 859-872), the proofs of the review written jointly with Drs. Bélanger and Greulich for the Annals of the New York Academy of Sciences have been corrected, while the article on the entry of Ca^{45} into growing teeth submitted to the Journal of Dental Research should appear in the course of this year.

Radioautographic Localization of Radio Carbon in
the Bones and Teeth of Newborn Rats After Administration of
 C^{14} Glucose

Y. Kumamoto and C.P. Leblond
Department of Anatomy, McGill.

A radioautographic study of the incorporation of C^{14} labelled glucose into teeth and other tissues of newborn rats was attempted. Studies with C^{14} -bicarbonate conducted by Greulich ('53, '54) had shown rapid and extensive incorporation into organs and tissues of young rats. It was of interest to find out if there would be a similar utilization of glucose and, if so, what the exact sites of deposition of C^{14} -glucose derivatives and/or products would be. This report is that of a survey of the sites of deposition of C^{14} in newborn rat tissues and especially in teeth after administration as glucose.

Materials and methods

Radioactive glucose labelled uniformly and endowed with a specific activity of 2.4 $\mu\text{c}/\text{mg}$ was supplied by Dr. G. Krotkov of Queen's University. A 10% solution of the glucose in distilled water was injected with a syringe fitted with a number 26 hypodermic needle. The injection was made through the abdominal wall into the stomach. Three newborn rats (5 grams) were each given 30 μc of C^{14} -glucose in a volume of 0.13 cc in this manner. No untoward effects were noticed after administration. The rats were killed after anesthesia at 3 $\frac{1}{2}$, 27 $\frac{1}{2}$, and 72 hours after injection. Teeth and other tissues were removed and fixed without delay in alcohol-formaldehyde, Orth and Carnoy. Paraffin sections were prepared for radioautography. Kodak NTB² emulsion was used in the coated method.

Results

Teeth. The amount of dentin and enamel present in molar teeth of the young rats used in this experiment was extremely small so that autographic localization of C^{14} within the dentin and enamel could not be accurately defined, although it predominated at the dentino-enamel junction. The intensity appeared somewhat greater over the dentin than over the enamel.

In the incisor teeth, more dentin and enamel were present. At the early time interval, maximum intensity of the autographic reactions were found over the predentin and reactions of lesser intensity were found over the dentin. Where the predentin appeared to be just forming, the autographic reaction could not be accurately localized to predentin or to the apical poles of

the odontoblasts. These cells were slightly more reactive than the cells of the pulp. At 27 $\frac{1}{2}$ hours after introduction of the isotope, the reaction was found in the new layers of dentinal matrix adjacent to the predentin and a reaction decreasing in intensity therefrom was found over the predentin. At 72 hours, the reaction was found well within the dentin (Fig. 1) and predentin did not show reactions of any significance. Intensity of cellular reactions, including those from the odontoblasts decreased by this time interval.

Autographic reactions over the enamel were light and diffuse throughout. The ameloblasts showed a somewhat greater reaction about their apical poles.

Proximal half of the tibia including the cartilage.

Hyaline cartilage of the head region of the tibia reacted lightly, decreasing slightly in intensity with time. The reaction was not clearly localized. In the zone of proliferating and seriated chondrocytes, reactions were found to be very intense and localized over the cells at 3 $\frac{1}{2}$ hours after the injection (Fig. 2) while the matrix about these cells was more reactive than that of the head region but much less so than the cells. By the third time interval, the cells were no longer as intensely reactive as in the earlier time interval, while the matrix appeared only slightly reduced in intensity. It was of interest to note that by the second time interval, when the loss of reaction from the chondrocytes became visible, that this loss began from the circumference of the epiphyseal region. In the zone of degenerating cells, the autographic reaction was almost entirely lost from the cells. The organic matrix also retained only a very small degree of reaction. The calcifying cartilage and bony trabeculae of the metaphyseal region contained greater reactivity than the immediately preceding zone. Reactivity appeared to emanate from all layers of the spicules at the early intervals but at later intervals, some trabeculae seemed to show peripheral, i.e., surface, deposition of C¹⁴. The intensity of the reaction decreased with time. The diaphysis, which is being formed showed autographic reactions mainly in the periosteal regions and at the 3 $\frac{1}{2}$ hour interval, it was hard to distinguish whether the reaction was from the periosteal cells (osteoblasts and fibroblasts) or from newly formed ossein.

Soft tissue. Representative tissues from organs throughout the body showed uptake of radiocarbon. Mucous cells of the submaxillary salivary gland, and the goblet cells of the intestine showed intense autographic reactions at 3 $\frac{1}{2}$ hour interval. Hibernating gland, which during the time interval of this experiment undergoes a rapid development, is found to be more reactive than other soft tissues at this time interval. At 27 $\frac{1}{2}$ hours after administration of C¹⁴-glucose, local-

ized reactions of the mucous containing cells were no longer present. The hibernating gland also lost its reactivity. On the whole though, reactions obtained for most of the tissues were slightly greater in intensity at this time interval than at the $3\frac{1}{2}$ hour interval. At 72 hours, the intensity of the reactions was much reduced.

Discussion

Since all tissues and organs are growing rapidly, all showed uptake of radioactivity when labelled glucose was administered intragastrically. The amount and rate of incorporation was greater in some structures than in others. Predentin, chondrocytes, mucous containing cells of the salivary gland and intestine showed rapid uptake. The reactions in the dentin and bone were permanent while those in the soft tissues were not during the experimental period.

The autographic results of soft tissues can be divided into two broad groups: those in which a maximum intensity was reached within $3\frac{1}{2}$ hours followed by a decrease and the other groups in which a generalized reaction decreased in intensity during the experimental period.

A comparison of the rate of organic turnover of different structures in a rapidly growing rat can thus be obtained in the group showing maximum incorporation of C^{14} within a short time interval. The decrease in autographic intensity observed in tissues of the second group may be due to either a loss of radioactive substance or to an addition of non-radioactive substances, and hence a lowering of specific activity. Since the organ is growing rapidly, there is new material constantly being built into it, and since the radiosugar is available in maximum amounts at one instant only, namely, at the time of injection or closely following it, subsequent growth cannot be visualized autographically. The quantitative aspects cannot be derived from this experiment.

Conclusion

Radioactive glucose administered intragastrically was found distributed in teeth, bones and soft tissues. Reactions in the dentin and bone were most permanent during the experimental period.

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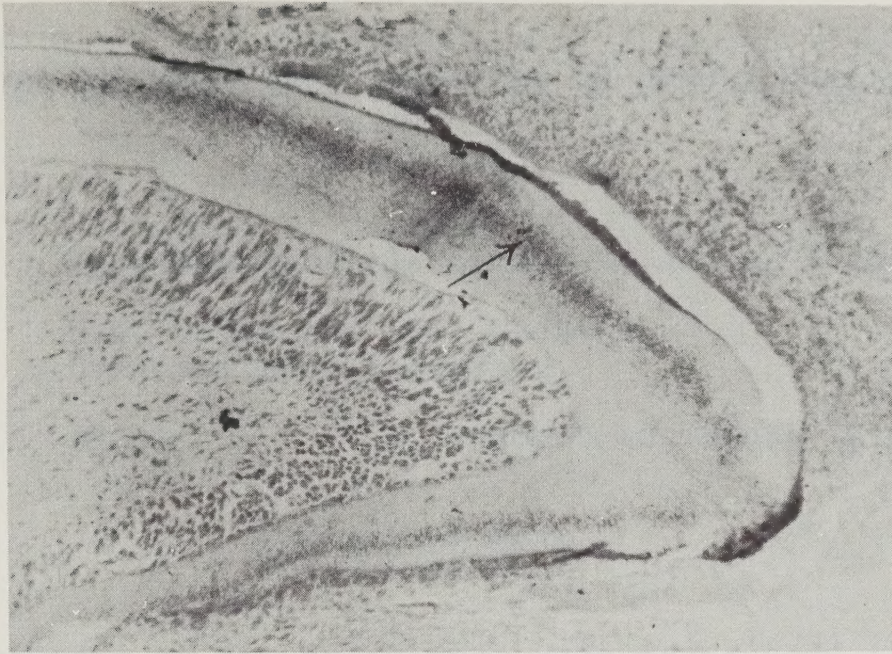


Fig. 1 Coated radio-autograph of upper incisor 72 hours after intragastric injection of C14-glucose to newborn rat. The site of deposition of C14 in the dentin is indicated by an arrow. H & E stain. x135 Kodak NTB2 emulsion.

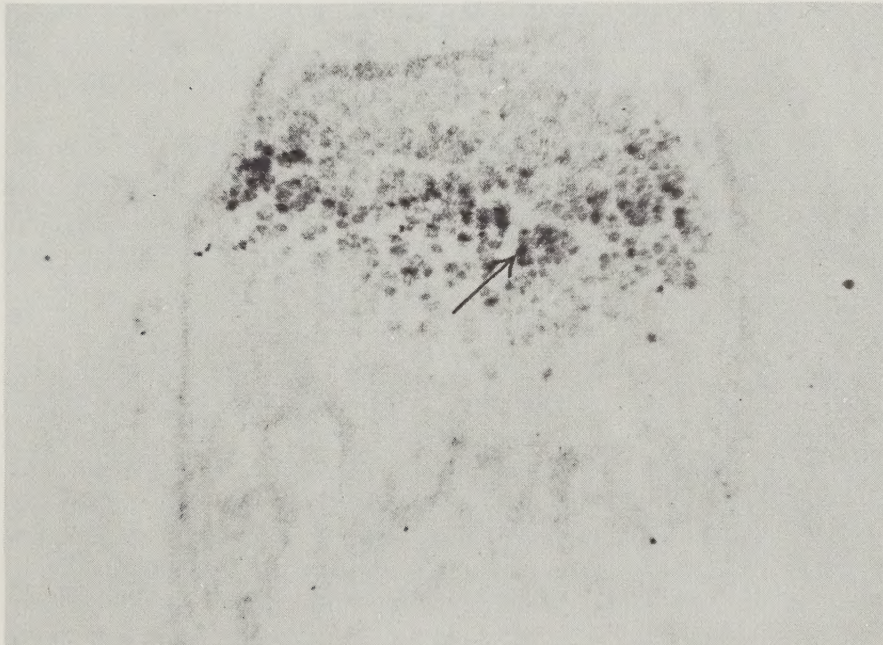


Fig. 2. Coated radio-autograph of head of tibia 3 1/2 hours after injection of radio-carbon to newborn rat. Radioautographic reactions are noted over the cartilage and chondrocytes (arrow). The zone of degenerating cells are hardly reactive, while the trabeculae show very light reaction. Unstained section. x80. Kodak NTB2 emulsion.

APPENDIX I

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Electropotentials caused by dental alloys.

Grantee: S.G. Davis and H.R. MacLean,
University of Alberta.

Project No.: DR-43

Amount of Grant: \$250.

SUMMARY OF WORK

Measurements of potential were made using gold alloys along with silver alloys. However, it was found necessary to increase the accuracy of the potential measurement. For this reason the condenser of the ballistic galvanometer meter was placed in a thermostat and maintained at a temperature of $27 \pm 0.1^{\circ}\text{C}$. The instrument was recalibrated after it was thermostated and this is now used.

The part-time services of a student have been obtained for February and March.

This report will give the analytical data on the teeth analysed during the past year and the means of all teeth in each group analysed since the study commenced. There are now fluorine figures on dentin and enamel for several hundred teeth; the statistical handling of the data could not be attempted this year. In this connection it might be noted that such an analysis may be complicated if it is necessary to remove differences due to different operators on the fluorine stills. This was found to be necessary on the study of the "Pabulum" and "non-Pabulum" teeth. It has been observed during the past six months that Miss Lin (who is carrying out the chemical analysis) is getting what appears to be lower fluorine percentages. Her "blank" determinations also are lower than any before obtained. Techniques and solutions are being scrutinized but as yet no reason for these lower results has been discovered and they are included in the means.

Tables I and II contain the results for the deciduous teeth from Brantford analyzed this year. In Table III the

APPENDIX J

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Fluoride content of dentin and enamel of teeth from Sarnia, Brantford and Stratford.

Grantee: Dr. M. Doreen Smith,
University of Toronto.

Project No.: DR-13

Amount of Grant: \$1,625.

ANNUAL REPORT

This is a continuation of the study of the fluorine content of teeth from the three Ontario areas used in evaluating water fluoridation. For some years permanent teeth have been received from Brantford (water 1 p.p.m. fluorine since 1945), Stratford (1.3 p.p.m. fluorine naturally) and Sarnia (no fluorine). Some deciduous teeth have come in from Brantford and the analysis of the enamel and dentin of these teeth in relation to time of exposure to fluoridated water has been quite interesting. It is regrettable that no deciduous teeth have been received from Stratford or Sarnia. In the past year 33 permanent teeth have been received from Brantford and 55 deciduous teeth, also 2 permanent teeth from Sarnia, all of which have been analysed for fluorine content of dentin and enamel.

This report will give the analytical data on the teeth analysed during the past year and the means of all teeth in each group analysed since the study commenced. There are now fluorine figures on dentin and enamel for several hundred teeth; the statistical handling of the data could not be attempted this year. In this connection it might be noted that such an analysis may be complicated if it is necessary to remove differences due to different operators on the fluorine stills. This was found to be necessary on the study of the "Pabulum" and "non-Pabulum" teeth. It has been observed during the past six months that Miss Lin (who is carrying out the chemical analysis) is getting what appear to be lower fluorine percentages. Her "blank" determinations also are lower than any before obtained. Techniques and solutions are being scrutinized but as yet no reason for these lower results has been discovered and they are included in the means.

Tables I and II contain the results for the deciduous teeth from Brantford analysed this year. In Table III the

means are given for all the deciduous teeth analysed from Brantford. When the average results from these teeth are examined it can be seen that the percentage of fluorine in both dentin and enamel is higher in the teeth of children who have been exposed to fluoridated water from birth (8 years or under) than in those from children exposed only 3 years or than children 9 years of age and older exposed for 7.5 to 9 years. The mean fluorine value in the dentin of this latter group is higher however than that of the dentin of teeth from children exposed only three years. These figures can be discussed more satisfactorily when a statistical analysis is made on them, although we have no control deciduous teeth from Sarnia or Stratford, as has already been noted.

Table IV gives the results of the permanent teeth from Brantford analysed this year and Table V the means of these figures combined with previous results of Brantford teeth with 8 years exposure and compared with the means results of teeth with only 3 years exposure to fluoridated water and the means of results of teeth from Sarnia and Stratford. The trends of fluorine accumulation are in the same direction as previously reported.

While this project has been concentrated on absorption or adsorption of fluorine by dentin and enamel from ingested water-borne fluorine, a secondary interest has been the sorption of fluorine by dental tissues from a less soluble form as is found in bonemeal. A study previously reported was made of this effect by comparing the teeth of children fed Pablum^{*} during infancy with the teeth of those fed no Pablum. The results from approximately 30 teeth in each group were examined statistically and checked by Mr. D.B.W. Redi of the Department of Biometrics, University of Toronto. The D/E ratios were significantly different at the 10% level, the Pablum teeth having the smaller ratio, which suggests more fluorine in the enamel of these teeth. This difference was not great but it was felt that the results were weighted in favour of the non-Pablum teeth as many children who said that they had been fed Pablum may have had only a small amount. When the Government of Newfoundland decided that all flour used in that country should have not less than 0.5% bonemeal added, it seemed an excellent opportunity to study the effect of this source of fluorine with respect to sorption by the teeth.

Table VI shows the results of analyses made on pooled teeth obtained from Newfoundland children who had been

* 2% bonemeal has always been added to Pablum until 1954 when this practise was discontinued.

ingesting this flour for about three years. Contacts have been made to obtain more teeth with the accompanying data but as yet none have been received.

In the interim report presented in September 1954, reference was made to a study of nutritional data and dental health data recorded by Dr. and Mrs. Pownall on 282 children. A statistical analysis has been applied to these data. After studying the nutrition records carefully they were sorted into three groups, "good", "fair" and "poor". In general those in the "good nutrition" group had vitamin C and D supplements and all had some citrus fruit. Mild, fish or meat, eggs or cheese were eaten daily or several times a week. About 23% of this group had no pastry and 19% no candy. In the group labelled "poor nutrition" very few had vitamin supplements or milk daily, 28% had no citrus fruit, most had meat or fish not as frequently as the "good nutrition" group. Most of them had no cheese and 11% no eggs; 8% had no cake or pastry daily and 10% had no candy.

The dental caries records of the children grouped as having "good" nutrition was compared by a statistical group comparison test with the dental caries records of the group classed as having "poor" nutrition. There were 88 children in the first group and 86 in the second. Table VII shows the results of the group comparison. The number of caries surfaces divided by the number of teeth in the mouth was used to measure the dental caries.

The mean for the "poor nutrition" group is significantly higher than that for the "good" group. Since the background and other conditions for these children were similar, it appears that the difference in dietary habits account for the difference in carious surfaces. This offers some additional evidence for an idea that is already fairly generally accepted. The results are included for the information of the Committee.

3 years' exposure to fluoridated water. Ages 5 - 12

15

.0125

.0042

2.50

8 - 9 years' exposure to fluoridated water Ages 4-9

41

.0125

.0042

2.50

43

.0125

.0042

2.50

Table I

Fluorine content of Brantford teeth from children born after fluoridation

Age	No. of teeth pooled	Type	<u>Deciduous</u>		D/E
			%fluorine in dentin	%fluorine in enamel	
4	1	Molar	.0150	.0069	1.22
5	2	"	.0251	.0146	1.72
5	2	"	.0321	.0188	1.71
5	1	"	.0406	.0250	1.62
5	1	"	.0135	.0033	4.09
5	1	"	.0248	.0048	5.16
5	1	"	.0208	.0042	4.95
7	1	"	.0209	.0057	3.66
7	2	"	.0376	.0100	3.76
7	2	Incisor	.0423	.0182	2.32
7	2	Molar	.0211	.0071	2.97
7	2	"	.0138	.0058	2.38
8	1	Cuspid	.0244	.0106	2.30
8	3	Molar	.0274	.0043	6.37
8	1	"	.0270	.0059	4.57
8	2	"	.0188	.0067	2.80
8	2	Cuspid	.0166	.0118	1.40
8	1	"	.0209	.0069	3.03
Mean	41*		.0272	.0105	3.02

* Including results of 13 teeth in this group previously reported in March, 1954.

Table II

Fluorine content of Brantford deciduous teeth from children born before fluoridation

Age	No. of teeth pooled	Type	% fluorine in dentin	% fluorine in enamel	D/E
9	1	Molar	.0174	.0039	4.45
9	1	"	.0231	.0047	4.91
9	3	Incisor	.0166	.0061	2.72
10	1	Molar	.0350	.0125	2.80
10	1	"	.0121	.0030	4.03
10	1	"	.0144	.0030	4.08
10	2	Cuspid	.0218	.0049	4.45
11	1	Molar	.0309	.0075	4.12
11	2	"	.0117	.0045	2.60
11	2	Cuspid	.0066	.0044	1.50
11	1	Molar	.0089	.0033	2.69
11	1	"	.0163	.0028	5.82
11	2	"	.0146	.0038	3.84
12	2	"	.0167	.0024	6.95
12	1	"	.0192	-	-
12	1	"	.0060	.0028	2.14
12	2	"	.0170	.0036	4.72
12	2	"	.0061	.0028	2.18
Mean	49*		.0214	.0062	4.39

* Including results of 22 teeth in this group previously reported in March, 1954.

Table III

Mean value of fluorine content in Brantford deciduous teeth by years of exposure

	No. of teeth	Av. % flu- orine in dentin	Av. % flu- orine in en- amel	D/E
3 years' exposure to fluoridated water. Ages 5 - 12	15	.0126	.0082	2.50
8 - 9 years' exposure to fluoridated water	41	.0272	.0105	3.02
Ages 9-12	49	.0214	.0062	4.39

Table IV

Fluorine content in Brantford permanent teeth extracted
8 - 9 years after fluoridation

Age	No. of teeth pooled	Type	% fluorine in dentin	% fluorine in enamel	D/E
7	1	Incisor	.0394	.0200	1.87
9	1	1st bicuspid	.0262	.0113	2.32
9	1	Molar	.0110	.0040	2.75
9	1	Bicuspid	.0282	.0112	2.52
9	1	"	.0226	.0085	2.66
10	1	Molar	.0287	.0044	6.52
10	2	Bicuspid	.0113	.0051	2.22
10	1	"	.0218	.0080	2.72
10	1	Incisor	.0135	.0058	2.33
10	2	1st bicuspid	.0164	.0081	2.02
10	1	1st molar	.0210	.0080	2.62
10	1	1st bicuspid	.0153	.0052	2.94
10	1	Bicuspid	.0132	.0063	2.09
10	1	"	.0130	.0060	2.16
10	1	"	.0165	.0076	2.17
11	1	1st molar	.0228	.0044	5.10
11	1	Incisor	.0157	.0063	2.49
11	1	Molar	.0123	.0038	3.24
11	1	Bicuspid	.0155	.0068	3.28
12	1	"	.0220	.0058	3.79
12	1	"	.0279	.0070	3.98
13	1	1st bicuspid	.0196	.0058	3.38
13	1	Molar	.0225	.0072	3.12
13	1	"	.0236	.0045	5.24
13	2	Bicuspid	.0092	.0028	3.29
13	1	"	.0128	.0023	5.56
14	1	2nd molar	.0110	.0020	5.50
14	1	Molar	.0240	.0037	6.48
15	1	1st molar	.0228	.0023	9.91
15	1	Molar	.0201	.0050	4.02
Mean	67*		.0283	.0098	3.63

* Including results of 34 teeth previously reported in
March, 1954.

Table V

Mean values of fluorine content in childrens' permanent teeth (up to 17 years of age).

	No. of teeth	Av. % F. in dentin	Av. % F. in enamel	D/E
Sarnia	26	.0101	.0047	2.7
Brantford	3 yrs. exposure 15	.0167	.0084	2.7
	8 yrs. exposure 67	.0283	.0098	3.6
Stratford	11	.0325	.0128	2.9

Percent fluorine in non-carious permanent teeth from Newfoundland

4.12	.0077	.0317	Cuspid
2.79	.0038	.0106	
2.79	.0038	.0106	
Mean 3.23	.0051	.0176	Mean

Fluorine content in the white wheat flour of Newfoundland - 2.8 p.p.m. fluorine.

Table VI

Percent fluorine in carious teeth from Newfoundland

Pooled Deciduous Teeth

Type	% fluorine in dentin	Type mean	% fluorine in enamel	Type mean	D/E
Incisor	.0219		.0056		3.91
	.0141	.0180	.0043	.0050	3.28
Cuspid	.0205		.0061		3.36
	.0155	.0180	.0024	.0042	6.74
1st molar	.0231		.0060		3.85
	.0139	.0185	.0028	.0044	4.96
2nd molar	.0101		.0032		3.16
	.0124	.0113	.0025	.0029	4.96
Mean	.0164		.0041		4.25

Pooled Permanent Teeth

Incisor	.0160		.0070		2.29
	.0421		.0058		7.25
	.0172		.0083		2.07
	.0078	.0208	.0032	.0061	2.44
Cuspid	.0432		.0108		4.00
	.0486		.0100		4.86
	.0142	.0353	.0046	.0085	3.08
Bicuspid	.0281		.0075		3.74
	.0141		.0050		2.42
2nd Bicuspid	.0146	.0189	.0053	.0059	2.75
1st molar	.0170		.0043		3.95
	.0176		.0088		2.00
	.0146	.0164	.0034	.0055	4.29
2nd Molar	.0112		.0038		2.95
	.0258		.0070		3.69
	.0316		.0044		7.18
	.0148	.0209	.0046	.0050	3.22
Mean	.0223		.0061		3.66

Percent fluorine in non-carious permanent
teeth from Newfoundland

Cuspid	.0317	.0077	4.12
	.0106	.0038	2.79
	.0106	.0038	2.79
Mean	.0176	.0051	3.23

Fluorine content in the white wheat flour of Newfoundland
- 2.8 p.p.m. fluorine.

Table VII

Mean caries figures of Newfoundland children aged 2 to 17 years

Nutrition group	No. of children	Mean of no. of caries surfaces per tooth
"Good"	86	$.41 \pm .03$ II
"Poor"	88	$.49 \pm .04$ *

* Standard error

Calculated $t = 2.44$

Tabulated $t_{.05} = 1.97$

APPENDIX K

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Comparative study of palatal height, width and length in ancient and modern crania.

Grantee: Dr. D.J.E. Mitchell,
University of Toronto.

Project No.: DR-56 Amount of Grant: \$1,870.

Project No.: DR-56 Amount of Grant: \$400.

SUMMARY OF WORK

Following the report made in October 1954, tooth injection by the suction method has been continued and experiments commenced. Statistical assessment of the data obtained in April 1954 at the New York Museum of Natural History indicates the necessity of larger sample sizes and greater racial variety. Palatal breadth appears significantly greater in primitive groups, whereas height and length seem to be similar to that of modern crania.

Arrangements have been made to examine Smithsonian collection of human crania in order to augment the data already obtained. It has also been decided to isolate the injected dental pulp by micro-dissection prior to making micro-arteriograms. The accompanying micro-arteriograms show the intra-dental and peri-dental vascular net in the entire tooth jaw and also in the isolated dental unit.

Unfortunately our X-ray tube is unsuitable for detailed studies such as this since it has a coarse focal spot (1 mm. sq.). We are accordingly unable to obtain highly resolved micro-radiographs and are conscious that we are losing a great deal of detail. This loss of detail is evident in Fig. 3.

It is my opinion that given a micro-focus unit with a fine focal spot (.04 mm.) and a range of 5 to 70 kv., we should be able to secure not only improved micro-radiographs but also obtain far more tissue detail, and be able to study features of internal structure invisible to the ordinary optical microscope. To obtain maximum contrast and tissue detail it is necessary to use an X-ray beam composed of the shortest (hardest) X-ray wave lengths and work at much lower kilovoltages than is possible with the available apparatus. Moreover it is important to secure initial enlargement of the specimen in the micro-radiograph before proceeding to enlargement by optical methods. This demands the

APPENDIX L

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Study of human dental pulp blood vessels by X-ray micro-arteriographic methods.

Grantee: Dr. R. L. deC. H. Saunders,
Dalhousie University.

Project No.: DR-51

Amount of Grant: \$1,870.

SUMMARY OF WORK

Following the report made in October 1954, tooth injection by the suction method has been continued and experiments commenced with the new chelating agents (Nikiforuk). Further confirmatory studies using histological methods are also in progress.

An introductory paper on the blood vessels of human dental pulp has been completed.

A series of foetal jaws of various ages have been injected and are being studied by microradiographic methods. It has also been found possible to isolate the injected dental sac by micro-dissection prior to making micro-arteriograms. The accompanying micro-arteriograms show the intra-dental and peri-dental vascular net in the entire foetal jaw and also in the isolated dental sac.

Unfortunately our X-ray tube is unsuitable for detailed studies such as this since it has a coarse focal spot (1 mm. sq.). We are accordingly unable to obtain highly resolved microradiographs and are conscious that we are losing a great deal of detail. This loss of detail is evident in Fig. 5.

It is my opinion that given a microfocus unit with a fine focal spot (.04 mm.) and a range of 5 to 50 kv, we should be able to secure not only improved microradiographs but also obtain far more tissue detail, and be able to study features of internal structure invisible to the ordinary optical microscope. To obtain maximum contrast and tissue detail it is necessary to use an X-ray beam composed of the softest (longest) X-ray wave lengths and work at much lower kilovoltages than is possible with the available apparatus. Moreover it is important to secure initial enlargement of the specimen in the microradiograph before proceeding to enlargement by optical methods. This demands the

use of an extremely fine focal spot and an increase in the object - film distance. At present we are limited to contact microradiography.

It should be evident from some of the illustrations (Figs. 2 & 5) that it is important that we be able to study the detail of the developmental progress of the intra-dental and peri-dental vascular nets, while observing concomitant changes in both the tooth and the immediately surrounding structures.

The development of a simple and rapid method of stereomicro-radiography shortly to be reported (Saunders, R.L.deC.H. and Lawrence, J., B.Sc.) has eliminated difficulties of interpretation occasioned by superimposition of vascular shadows.

An introductory paper on some of the developmental aspects of the human dental pulp vessels is now in the process of preparation. In regard to both papers I prefer to use the term introductory since a comprehensive statement of the vasculature of the developing and adult tooth must be regarded as a long term project requiring the study of a considerable amount of material.

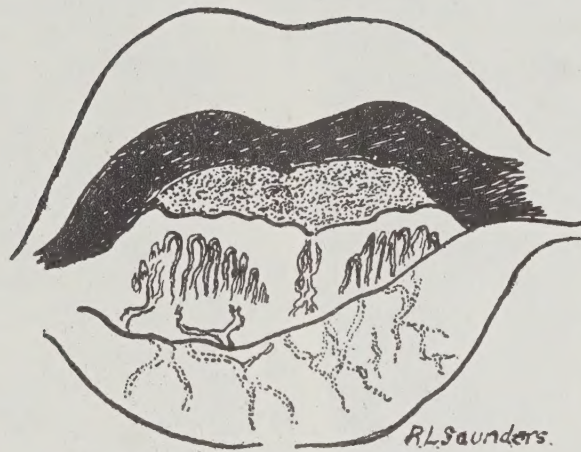


Fig. 1. Injection Telltale. Sketch showing appearance in gum and lip indicative of satisfactory injection of foetal jaw. Note vascular loops forming a crescent in the gum; also the symphyseal vessels in the midline. Foetus. C.R.10.6cm. Age 4 months.

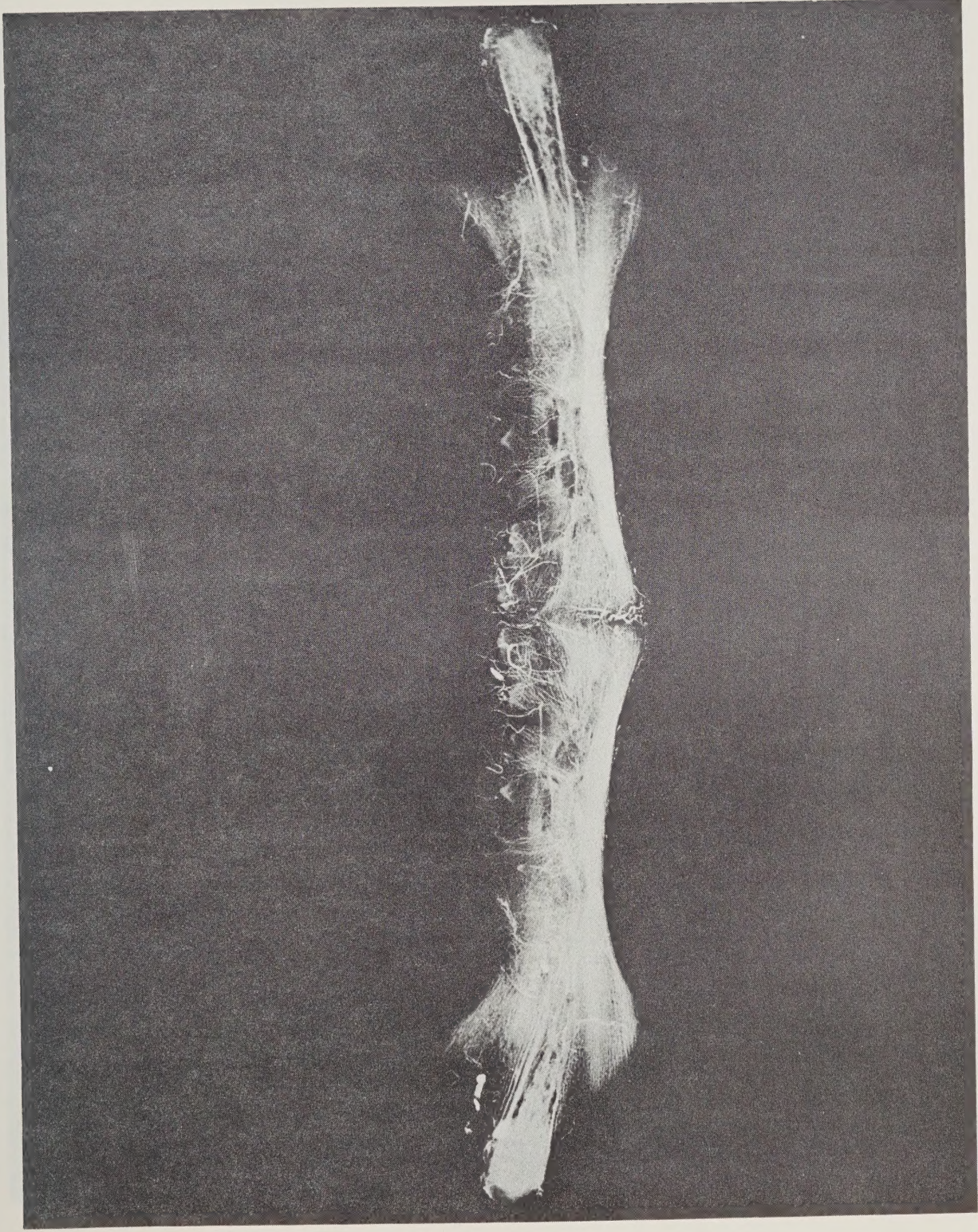


Fig. 2. Microradiograph of Foetal Mandible. Observe the peridental vascular net surrounding the primordia of the deciduous incisors, cuspids and molars. Foetus.C.R.21.8 cm. Age 6 months.



Fig. 3. Microradiograph of Foetal Maxilla, showing primordia of upper central incisors, which are seen as transverse white (radiopaque) streaks at the lower centre of the plate. Note the peridental net about each. The other dental primordia are largely obscured by the maxillary structures.



Fig. 4. Microarteriogram showing the intradental plexus beneath each dental primordium, within the dental papilla. Observe the continuity of this plexus with the peridental plexus on the enveloping dental sac, which has been resolved by stereo-microradiography. These plexuses are conceivably related to the process of dental eruption.

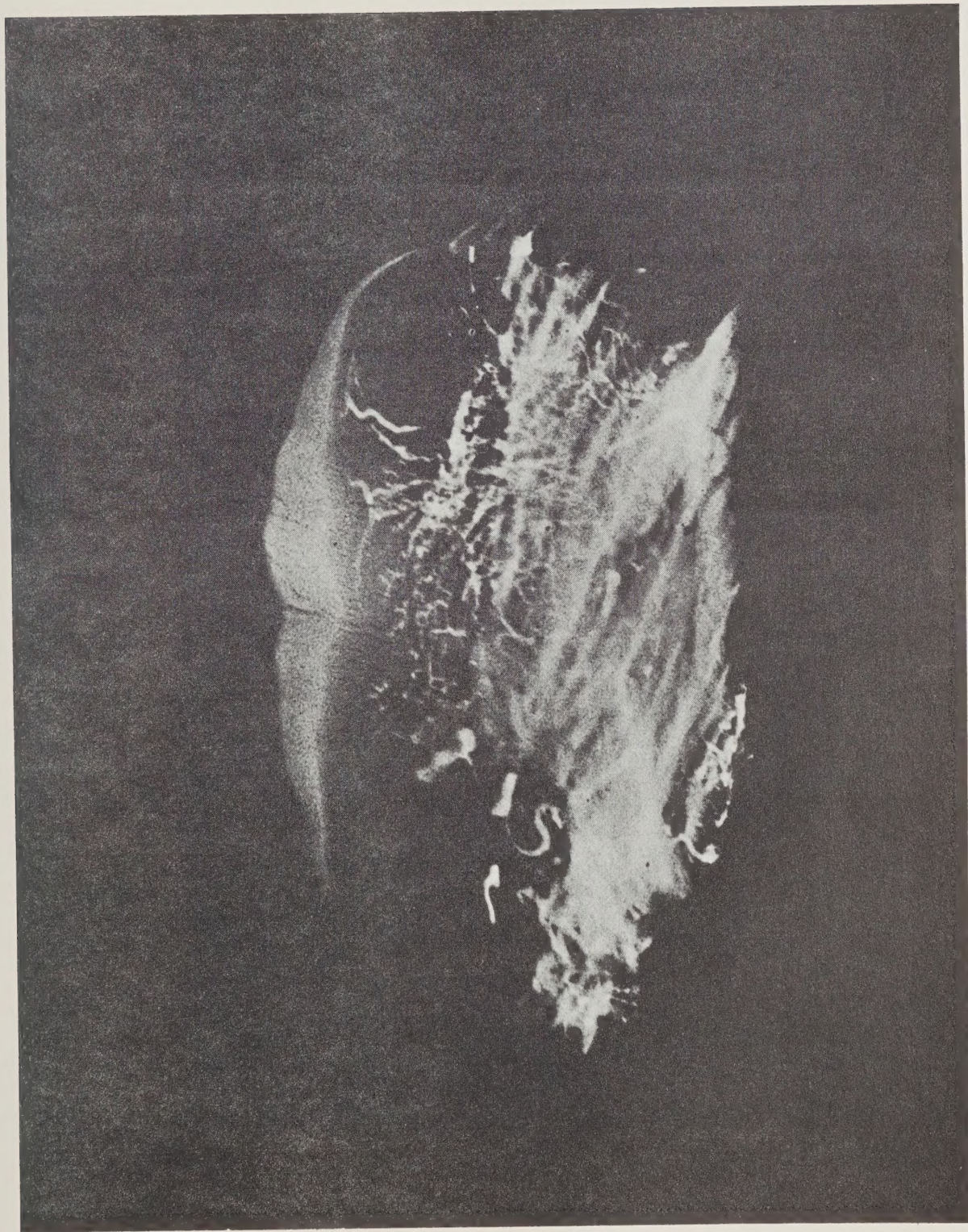


Fig. 5. Isolated primordium of upper central incisor following removal of upper half of dental sac. From above downwards note the enamel cap, intradental vascular plexus within the dental papilla, and lastly a few bony trabeculae obscuring the lower part of the plexus. Foetus. C.R.21.8 cm. Age 6 months.

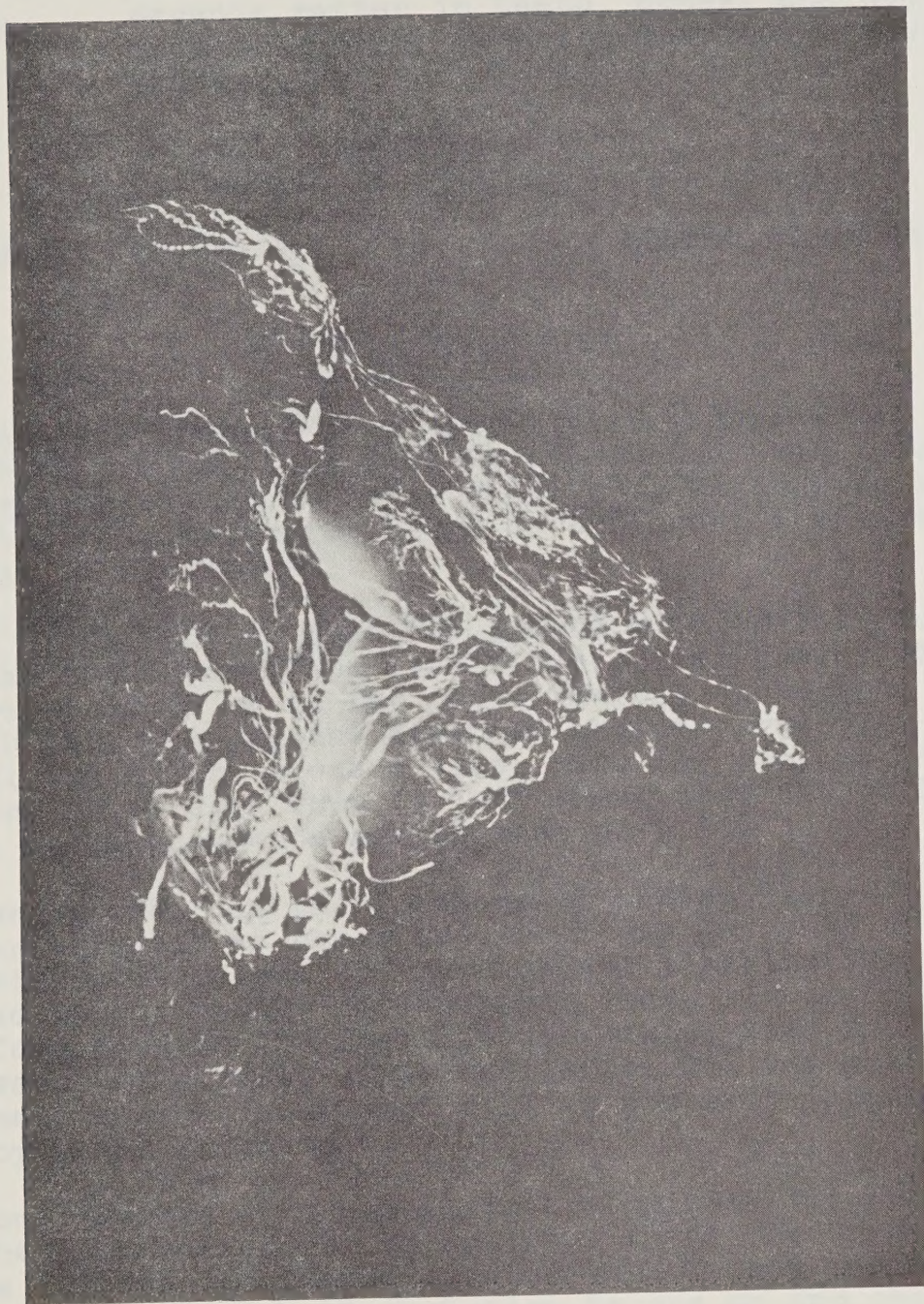


Fig. 6. Microarteriogram showing two isolated dental primordia, with infradental and peridental vascular plexuses.

APPENDIX M

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: Investigation of methods for destroying the organic matter in teeth, bones, and other materials prior to determination of fluorine content.

Grantee: Dr. O. J. Walker,
University of Alberta.

Project No.: DR-28 Amount of Grant: nil

SUMMARY OF WORK

I regret to report that very little progress has been made on this project this past twelve months even though I had a research assistant, Miss Hinda Doz, anxious to get along with the problem for eight or ten hours a week since the middle of September.

The difficulty is that the Lumetron Photoelectric colorimeter has been undergoing repair since last March. We have had it returned to us on two occasions but found that repairs were such that the machine does not operate properly. This is the condition at the present time, with the company saying they found it to work when it left their shop and with us finding that no measurements can be made with it.

However, we felt that methods of ashing fluoride containing materials such as bones and teeth can be ashed successfully, without the loss of fluorine, by alkaline reagents as described in our report of September 1953. In order to get more information on the distillation stage, Miss Doz has made about twenty-five runs. The distillates are being stored until such time when the instrument will be properly repaired or replaced by a new instrument.

Some members of the Associate Committee are probably not aware that the method for determining fluorides used by us requires a fifteen centimetre cuvette rather than one in which the light path through the solution is one or two centimetres as in most determinations using a photoelectric colorimeter.

APPENDIX N

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1955

Subject: A cephalometric study of maxillary growth in relation to statural growth.

Grantee: Dr. M.R. Culbert,
University of Toronto.

ProjectNo.: DR-55 Amount of Grant: \$175.

SUMMARY OF WORK

Measurements of maxillary length were made from serial cephalometric profiles of sixty-three individuals (as reported in more detail previously), in the Higley-Meredith study at the University of Iowa. Statural growth data for each individual were recorded also.

These two sets of figures will be analysed and studied to determine if a definite co-relation exists.

APPENDIX O

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

List of Grantees and Subjects

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-1	Active	Dr. J. J. Rae, Dept. of Chemistry, University of Toronto	Chemical mechanisms in dental caries
DR-2	Completed	Dr. G.H.W. Lucas, Dept. of Pharmacology, University of Toronto	A study of alveolar oxygen tension and blood oxygen con- tent during nitrous oxide anaesthesia.
DR-3	Completed	Dr. H. K. Box Faculty of Dentistry, University of Toronto	A study of micro- organisms found in deep periodontal pockets and caries lesion as revealed by the electro- microscope.
DR-4	Completed	Dr. H. K. Box, Faculty of Dentistry, University of Toronto.	An investigation of packing material in the treatment of periodontal disease (continuation of in- vestigation previous- ly carried on by Maj. Linghorne)
DR-5	Completed	Major R. L. Twible, Royal Can. Dental Corps (later O.R.F.)	To improve certain properties of the acrylic resin den- ture base and to effect an economy in denture produc- tion.
DR-6	Completed	Brig. E.M. Wansbrough, Royal Can. Dental Corps	To study the effects of altitude acceler- ation and deceler- ation on oral tissues
DR-7	Completed	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries.

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-8	Completed	Dr. A.D.A. Mason, Faculty of Dentistry, University of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply.
DR-9	Completed	Dr. O.J. Walker, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, University of Alberta	An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry.
DR-10	Completed	Dr. G.M. Macdonald, Queen Mary's Veterans Hospital, Montreal	Facial prostheses.
DR-11	Completed	Dr. R.J. Godfrey, Faculty of Dentistry, University of Toronto	An anatomical, histological and physiological study of the role of the condyle in mastication.
DR-12	Completed	Dr. A.E.R. Westman, Ont. Research Foundation.	Investigation of cements for methacrylate inlays.
DR-13	Active	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Fluoride content of dentine, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford.
DR-14	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto.	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries.
DR-15	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto.	A comparison of the patterns of eruption of the deciduous teeth in man with rate of growth of the dental arches.

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-16	Completed	Dr. C.H. Best, Dept. of Medical Res., University of Toronto	Studies in vitro of certain fungus-like organisms found in periodontal diseases
DR-17	Completed	Dr. R.F. Shaner, Dept. of Anatomy, University of Alberta	An investigation of the effects of solutions used in operative dentistry on the vital pulps of teeth.
DR-18	Completed	Dr. H. K. Box, Faculty of Dentistry, University of Toronto	Experimental production of dental caries.
DR-19	Completed	Dr. E.A. Sellers, Dept. of Pharmacology, University of Toronto	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry
DR-20	Completed	Dr. Jules Thébaud, Faculty of Dental Surgery, University of Montreal	Improvement of subgingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris.
DR-21	Completed	Dr. A.E.R. Westman, O.R.F.	Investigation of repair techniques for methacrylate dentures.
DR-22	Active	Dr. C.H. Best, Dept. of Medical Res., University of Toronto	An investigation of the mechanism of repair of the supporting structures of the teeth and factors affecting the reparative process.
DR-23	Active	Dr. C. P. Leblond, Dept. of Anatomy, McGill University	Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.
DR-24	Completed	Dr. C.H.M. Williams, Faculty of Dentistry, University of Toronto	The effects of hard and soft diets on the supporting structures of the teeth.

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-25	Completed	Dr. A.E.R. Westman, O.R.F.	Investigation of methods for the use of methyl methacrylate in direct dental fillings.
DR-26	Completed	Dr. R.E. Moyers, Faculty of Dentistry, University of Toronto	A study of the normal and abnormal physiologic forces of the oral cavity.
DR-27	Completed	Dr. J.W. Abbiss, Faculty of Dentistry, Dr. A.G. Nutlay, Faculty of Dentistry, Dalhousie University	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth.
DR-28	Active	Dr. O.J. Walker, Dept. of Chemistry, University of Alberta	Investigation of methods for destroying the organic matter in teeth, bones and other materials prior to determination of fluorine content.
DR-29	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, University of Toronto	Biological absorption of fluorine.
DR-30	Completed	Dr. Norma Ford Walker, Dept. of Zoology, University of Toronto	The study of the closure of space following premature loss of deciduous teeth.
DR-31	Completed	Dr. L.B. Jaques, Dept. of Physiology, Mr. G.J. Millar, Dept. of Physiology, University of Saskatchewan	An investigation of nerve block.
DR-32	Completed	Dr. A. W. Ham, Dept. of Anatomy, University of Toronto	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structures of the growing incisor teeth of animals.

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-33	Completed	Dr. H. A. Hunter, Faculty of Dentistry, University of Toronto	A study of calcifying potential of calcium oleate on the dental pulp.
DR-34	Completed	Dr. A. D'Iorio, Dept. of Physiology, University of Montreal	Studies of calcium metabolism in teeth with the aid of Ca ⁴⁵
DR-35	Active	Dr. J.B. Macdonald, Div. of Dental Res., University of Toronto	Bacteriology of periodontal disease. I. Experimental fusospirochetal infection with mixtures of pure culture.
DR-36	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Demineralization of hard tissues as bone and teeth by organic chelating agents.
DR-37	Active	Dr. H. Jenkins, Dept. of Orthodontics, University of Toronto.	Further studies in the electrolyography of the head, face and neck.
DR-38	Completed	Dr. K.J. Paynter, Faculty of Dentistry, University of Toronto	The effects of the endocrines on the teeth and jaws. II. Further studies on the effect of thiouracil on the development of molar teeth of rats.
DR-39	Completed	Dr. A. G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs.
DR-40	Completed	Dr. L.A. Funt, Faculty of Dentistry, University of Toronto	Effect of tooth eruption and function on the growing of the mandible and facial complex on cats.

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-41	Completed	Dr. H. Jenkins Faculty of Dentistry, University of Toronto	A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions (After Downs (1))
DR-42	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators.
DR-43	Active	Dr. S. G. Davis, Dept. of Chemistry, Dr. H. R. MacLean, Faculty of Dentistry, University of Alberta	Electro potentials caused by dental alloys.
DR-44	Completed	Dr. A. H. Craven, Faculty of Dentistry, McGill University	Facial and cranial growth patterns in the rat as revealed by vital staining with alizarine red S.
DR-45	Completed	Dr. R. E. Moyers, Faculty of Dentistry, University of Toronto	Study of the incidence of malocclusion.
DR-46	Completed	Dr. H. Jenkins, Dept. of Orthodontics, University of Toronto	Study of treated cases.
DR-47	Completed	Dr. H.A. Hunter, Faculty of Dentistry, University of Toronto	The pathological characteristics of experimental fusospirochetal infections.
DR-48	Completed	Dr. G. Nikiforuk, Faculty of Dentistry, University of Toronto	The effect of topical application of silicones in protecting teeth against acid solutions in vitro and dental caries produced in vitro.

<u>Grant No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-49	Completed	Dr. A. H. Craven, Faculty of Dentistry, University of Toronto	A recording device to determine postural changes of the head in the horizontal plane.
DR-50	Active	Dr. J.B. Macdonald, Div. of Dental Res., University of Toronto	The cultivation and characteristics of <u>Bacteroides nigrescens</u>
DR-51	Active	Dr. R.L.deC.H.Saunders, Dept. of Anatomy, Dalhousie University	Study of human dental pulp blood vessels by X-ray microarteriographic methods.
DR-52	Active	Dr. L. F. Bélanger, Dept. of Histology and Embryology, University of Ottawa	Mechanism of bone and tooth formation in the light of autoradiography and histochemistry.
DR-53	Completed	Dr. C. B. Weld, Dept. of Physiology, Dalhousie University	The macrophages in the pulp tissue of the rat incisor and lower jaw.
DR-54	Active	Dr. B.N. Kropp, Faculty of Medicine, Queen's University	Development of a rapid method for grinding thin sections of plastic-embedded teeth.
DR-55	Active	Dr. M.R. Culbert, Dept. of Orthodontics, University of Toronto	A cephalometric study of maxillary growth as related to statural growth.
DR-56	Active	Dr. D.J.E. Mitchell, Dept. of Orthodontics, University of Toronto	Comparative study of palatal height, width and length in ancient and modern crania.
DR-57	Active	Dr. G. Nikiforuk, Div. of Dental Res., University of Toronto	The amino acid composition of enamel protein using paper chromatography

APPENDIX P

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Papers Published

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
1	New aspects of periodontal research, by H. K. BOX	Presented at National Convention of Can. Dentists, Toronto, May 29, 1946 (App. "C", 5th Mtg. A.C.D.R., 25 Feb. 1947)
2	Concerning the pathogenesis of periodontal disease, by H. K. BOX.	J. Periodontology 19: 11-20, 1948.
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C. T. CLEGG.	J. Dent. Res. 27: 52-53, 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J.J. RAE and C. T. CLEGG.	J. Dent. Res. 27: 54-57, 1948.
5	An improved method for processing acrylic dentures, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 14: 303-317, 1948.
6	The therapeutic properties of periodontal cement packs, by W. J. LINGHORNE and D.C. O'CONNELL.	J. Can. Dent. Assoc. 15: 199-205, 1949.
7	Phosphatase in saliva, by J. T. DENTAY and J. J. RAE.	J. Dent. Res., Feb. 1949.
8	The treatment of acute oral Vincent's infection, by W.J. LINGHORNE and D.F. STEDMAN.	J. Can. Dent. Assoc. July 1949.
9	A comparison of nine local anaesthetics, by H.S.HAMILTON B.A. WESTFALL and J.K.W. FERGUSON.	J. Pharmacol. Expt. Ther. 94: 299-307, 1948.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
10	New methods of repairing acrylic dentures without distortion, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 15: 582-590, 1949.
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. COGGLES, O.J. WALKER and H.R. MacLEAN.	J. Can. Dent. Assoc. 16: 75-82, 1950.
12	The relation between buffering capacity, viscosity and lactobacillus count of saliva, by J. J. RAE and C. T. CLEGG.	J. Dent. Res. 28: 589-593, 1949.
13	Mineralization of the growing tooth as shown by radio-phosphorus autographs (17692), by L. F. BELANGER and C. P. LEBLOND.	Proc. Soc. Expt. Biol. Med. 73: 390-391, 1950.
14	Radio-autographic visualization of bone formation in the rat, by C. P. LEBLOND, G. W. WILKINSON, L. F. BELANGER and J. ROBICHON.	Am. J. Anat. 86: 2, 1950
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH.	Can. J. Research, F, 28: 227-233, 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W. J. LINGHORNE and D. C. O'CONNELL.	J. Dent. Res. 29: 419-428, 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH.	J. Can. Dent. Assoc., Jan., 1951.
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J. P. LUSSIER.	Rev. Canadienne de Biol., 10: 175-180, 1951.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
19	Relining acrylic dentures without distortion, by D.R. CHRISTIE.	J. Can. Dent. Assoc., July, 1951.
20	Acrylic fillings - general comments and some experimental data, by D.R. CHRISTIE	J. Can. Dent. Assoc., 17: 427-435, 1951.
21	Ammonia production in saliva, by R. M. BALLANTYNE, C.T. CLEGG, J.J. RAE and F.H. LAWFORD.	J. Dent. Res. 30: 385-387, 1951.
22	Studies in the regeneration and reattachment of supporting structures of the teeth. II. Regeneration of alveolar process, by W. J. LINGHORNE and D.C. O'CONNELL	J. Dent. Res. 30: 604-614, 1951.
23	Radioactive isotopes in dental research, by J.P. LUSSIER and A. D'IORIO.	The Brit. Dent. Annual, 1952.
24	Determination of fluoride in water by the aluminum-hematoxylin method, by MARGARET J. PRICE and O. J. WALKER.	Anal. Chem. 24: 1593, 1952.
25	Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat, by L. F. BELANGER.	Science, 118: 520-521, 1954.
26	The motile non-sporulating anaerobic rods of the oral cavity, by J.B. MACDONALD.	University of Toronto Press, Toronto, 1953.
27	Motility in a species of non-flagellated bacteria (20677), by J.B. MACDONALD, M. L. KNOLL and R.M. SUTTON	Proc. Soc. Exp. Biol. & Med. 84: 459-462, 1953.
28	The periodontium and salivary glands in the alarm reaction, by G. SHKLAR and I. GLICKMAN.	J. Dent. Res. 32: 773-778, 1953.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
29	Autoradiographic visualization of S^{35} incorporation and turnover by the mucous glands of the gastro-intestinal tract and other soft tissues of rat and hamster, by L.F. BELANGER.	Anat. Rec. 118: 755-772, 1954.
30	Autoradiographic visualization of the entry and transit of S^{35} in cartilage, bone, and dentine of young rats and the effect of lyaluronidase in vitro, by L. F. BELANGER.	Can. J. Biochem. & Physiol. 32: 161-169, 1954.
31	Autoradiographic visualization with Ca^{45} of normal growth of the incisor of pigs and the effect of fluorine feeding, by L.F. BELANGER, W.E. LOTZ, W.J. VISEK and C.L. COMAR.	Anat. Rec. 119: 53-70, 1954.
32	Fluorine balance studies of four infants, by M.P. HAM and M. DOREEN SMITH.	J. Nutrition 53: 215-223, 1954.
33	Fluorine balance studies of three women, by MARY P. HAM and M. DOREEN SMITH.	J. Nutrition, 53: 225-232, 1954.
34	The effect of propylthiouracil on the development of molar teeth of rats, by K.J. PAYNTER.	J. Dent. Res. 33: 364-376 1954.

APPENDIX Q

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment

	<u>Term to Expire</u>
★ 1. Dr. J. B. Macdonald, <u>Chairman</u> Division of Dental Research, University of Toronto, Toronto, Ontario.	31 March 1957
2. Dr. E. W. R. Steacie, <u>ex officio</u> President, National Research Council, Ottawa 2, Canada.	- -
3. Dr. E. G. D. Murray, <u>ex officio</u> Dept. of Bacteriology and Immunology, McGill University, Montreal, P.Q.	- -

Representing the Dental Profession

4. Dr. J. S. Bagnall, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March 1955
5. Dr. C. D. Husband, Suite 110, Metro Block, Red Deer, Alberta.	31 March 1956
6. Dr. Jean-Paul Lussier, Faculty of Dental Surgery, University of Montreal, Montreal, P.Q.	31 March 1957
7. Dr. R. H. McDougall, 1505 Hampshire Road, Victoria, B.C.	31 March 1955
★ 8. Dr. A. G. Racey, Dept. of Oral Pathology, McGill University, Montreal, P.Q.	31 March 1956
9. Dr. C.H.M. Williams, Faculty of Dentistry, University of Toronto, Toronto 5, Ontario.	31 March 1957

★ Member of Executive

Representing the Royal Canadian Dental Corps,
Department of National Defence

- ★ 10. Brig. E.M. Wansbrough, ex officio,
Director General of Dental Services,
Royal Canadian Dental Corps,
Dept. of National Defence,
Ottawa, Ontario.

Representing the Division of Dental Health,
Dept. of National Health and Welfare

11. Dr. H.K. Brown, ex officio
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ontario.

Representing Dental Research,
Department of Veterans Affairs

12. Dr. L. A. Kilburn, ex officio,
Director of Dental Research,
Dept. of Veterans Affairs,
Ottawa, Ontario.

Representing the Basic and Medical Sciences

- | | |
|---|---------------|
| 13. Dr. J. G. Aldous,
Dept. of Pharmacology,
Dalhousie University,
Halifax, N.S. | 31 March 1956 |
| 14. Dr. H. J. Barrie,
Dept. of Pathology,
Faculty of Medicine,
University of Toronto,
Toronto, Ontario. | 31 March 1957 |
| 15. Dr. J. M.R. Beveridge,
Dept. of Biochemistry,
Queen's University,
Kingston, Ontario. | 31 March 1956 |
| 16. Dr. A. C. Burton,
Dept. of Biophysics,
University of Western Ontario,
London, Ontario. | 31 March 1956 |
| 17. Dr. D. H. Copp,
Dept. of Physiology,
Faculty of Medicine,
University of British Columbia,
Vancouver, B.C. | 31 March 1956 |

(1) To members of the

Term to Expire

★ 18. Dr. A. W. Ham, Committee on Dental Research 31 March 1957
 Dept. of Anatomy,
 Faculty of Medicine,
 University of Toronto,
 Toronto, Ontario.

19. Dr. Edouard Page,
 Director, Dept. of Nutrition,
 Medical School,
 Laval University,
 Quebec, P.Q.

31 March, 1955

★ 20. Dr. J. B. Marshall, Secretary,
 National Research Council,
 Ottawa, Canada.

- -

★ Member of Executive

(11) To other persons

21-23. Deans of Dental Schools (who are not members of the Committee)

21. Alberta - Dr. W. S. Hamilton

22. Dalhousie - Dr. J. D. McLean

23. McGill - Dr. D. Prescott Perry

24. Master Copy (General Secretary)

25. Board Room Copy

26. Library

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INITIAL DISTRIBUTION

(i) To members of the

Associate Committee on Dental Research

- | | |
|---|---|
| 1. Dr. J. B. Macdonald, <u>Chairman</u> | 11. Dr. L.A. Kilburn |
| 2. Dr. J.G. Aldous | 12. Dr. J.-P. Lussier |
| 3. Dr. J.S. Bagnall | 13. Dr. R.H. McDougall |
| 4. Dr. H. J. Barrie | 14. Dr. E.G.D. Murray |
| 5. Dr. J.M.R. Beveridge | 15. Dr. E. Page |
| 6. Dr. H.K. Brown | 16. Dr. A.G. Racey |
| 7. Dr. A.C. Burton | 17. Dr. E.W.R. Steacie |
| 8. Dr. D. H. Copp | 18. Brig. E.M. Wansbrough |
| 9. Dr. A. W. Ham | 19. Dr. C.H.M. Williams |
| 10. Dr. C. D. Husband | 20. Dr. J.B. Marshall
<u>Secretary</u> |

(ii) To other persons:

- 21-23 Deans of Dental Schools (who are not members of the Committee)
- | | | |
|---------------|---|-----------------------|
| 21. Alberta | - | Dr. W. S. Hamilton |
| 22. Dalhousie | - | Dr. J. D. McLean |
| 23. McGill | - | Dr. D. Prescott Mowry |
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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
FOURTEENTH MEETING
OF THE
EXECUTIVE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

5 MARCH 1956

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Fourteenth Meeting

of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in Room 411, Lord Elgin Hotel, at 8:00 p.m., March 5th, 1956.

1. Attendance

Dr. J.B. Macdonald, Chairman

Dr. A.W. Ham

Dr. A.G. Racey

Brig. E.M. Wansbrough

Dr. J.B. Marshall, Secretary

2. Applications for Grants

The Executive gave careful consideration to 14 applications for grants in aid of research totalling \$45,573. The following recommendations were made for presentation at the second session of the Committee on March 6th.

J.J. Rae, Dept. of Chemistry, Univ. of Toronto	(a) A bactericidal material in tooth enamel. (b) The chemistry of calcification. (DR-1) \$1,080.	NOT APPROVED
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C.H. Best, Banting & Best Dept. of Medical Research, Univ. of Toronto	An investigation of the mech- anism of repair of the supporting structures of the teeth. (DR-22) \$4,975.	APPROVED \$3,975.
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The Executive recommended that a Sub-Committee on Periodontal Diseases be set up to direct research in this field. It was felt that such a group might suggest to Dr. Linghorne that he review all the literature on periodontal regeneration

before proceeding with his project; the work with Dr. Macnab on bone repair is getting beyond the scope of the project for which the grant is made. It was agreed that Dr. Williams, Dr. Ham and Dr. Barrie would be most qualified to assist Dr. Linghorne in re-orienting his study.

C.P. Leblond,
Dept. of Anatomy,
McGill Univ.

Entry of carbohydrates into
teeth as shown with the help
of radioactive elements.
(DR-23) \$3,300.

APPROVED \$3,300.

R.L. de C.H. Saunders,
Dept. of Anatomy,
Dalhousie Univ.

A study of developing and
adult human intradental and
peridental vascular patterns
by microradiographic methods.
(DR-51) \$2,130.

APPROVED \$1,380.
(Freeze drying
equipment not
approved.)

L.F. Belanger,
Dept. of Histology,
Univ. of Ottawa

Mechanism of bone and tooth
formation.
(DR-52) \$2,465.

APPROVED \$2,465.

G. Nikiforuk,
Div. of Dental
Research,
Univ. of Toronto

Organic components of dental
tissues.
(DR-57) \$2,980.

APPROVED \$2,980.

J.G. Aldous,
Dept. of Pharma-
cology,
Dalhousie Univ.

Metabolism of procaine and
related compounds. III and IV.
(DR-58) \$3,000.

APPROVED \$3,000.

D.H. Copp,
Dept. of Physiology,
Univ. of British
Columbia

Phosphorus deficiency effects
on bones and teeth.
(DR-59) \$5,450.

APPROVED \$5,000.

F.C. Fraser,
Dept. of Genetics,
McGill Univ.

Studies on the pathogenesis
of cleft palate.
(DR-60) \$1,588.

APPROVED \$1,588.
(Dr. Fraser to be
directed to send
future applications
to Assisted Res-
earches Committee)

A.S.V. Burgen, Dept. of Physiology, McGill Univ.	Secretion of protein in saliva. (DR-61) \$5,950.	APPROVED \$3,400.
Recommended that Dr. Burgen be invited to attend next meeting of Committee to report on his work in view of lack of detail given in his report and application.		
K.J. Paynter, Div. of Dental Res., Univ. of Toronto	Investigations into the nature of cementum. \$3,000.	APPROVED \$3,000.
K.J. Paynter, Div. of Dental Res., Univ. of Toronto	Histological study of teeth and periodontal tissues of guinea pigs reared on sub- minimal allowances of ascorbic acid. \$1,455.	APPROVED \$1,455.
K.I. Melville, Dept. of Pharma- cology, McGill Univ.	Studies on the mechanism of gingival changes assoc- iated with drug adminis- tration \$5,600.	APPROVED \$3,000. (\$2,000 for salary of Dr. Francis and \$1,000 for supplies)
G. Pelletier, J.G. Perreault, Dept. of Dental Surgery, Univ. of Montreal	An evaluation of the dis- turbance of the dentine for- mation in the rat incisor following trauma of various origins \$2,600.	APPROVED \$2,600.

Applications for Fellowships

Three applications for Fellowships were reviewed by the Executive. The following recommendations were made for presentation at the second meeting of the Committee:

Dr. E.M. Madlener, Senior Dental Research Fellowship of \$4,000, to work under Dr. J.B. Macdonald, in the Division of Dental Research at the University of Toronto.

Dr. M. Weiss, Graduate Dental Research Fellowship of \$3,500, to work under Dr. A.S.V. Burgen in the Department of Physiology at McGill University.

Dr. A.M. Hunt, Graduate Dental Research Fellowship of \$3,000, to work under Dr. K.J. Paynter in the Division of Dental Research at the University of Toronto.

The meeting adjourned at 11:45 p.m.

INITIAL DISTRIBUTION

(i) To members of the

Associate Committee on Dental Research

- | | |
|--|--|
| 1. Dr. J.B. Macdonald, <u>Chairman</u> | 11. Dr. L.A. Kilburn |
| 2. Dr. J.G. Aldous | 12. Dr. J-P Lussier |
| 3. Dr. J.S. Bagnall | 13. Dr. E.G.D. Murray |
| 4. Dr. H.J. Barrie | 14. Dr. E. Page |
| 5. Dr. J.M.R. Beveridge | 15. Dr. A.G. Racey |
| 6. Dr. H.K. Brown | 16. Dr. E.W.R. Steacie |
| 7. Dr. A.C. Burton | 17. Brig. E.M. Wansbrough |
| 8. Dr. D.H. Copp | 18. Dr. C.H.M. Williams |
| 9. Dr. A.W. Ham | 19. Dr. J.B. Marshall,
<u>Secretary</u> |
| 10. Dr. C.D. Husband | |

(ii) To other persons:-

20-23 Deans of Dental Schools (who are not members of the
Committee)

- | | | | |
|-----|-----------|---|-----------------------|
| 20. | Alberta | - | Dr. W.S. Hamilton |
| 21. | Dalhousie | - | Dr. J.D. McLean |
| 22. | McGill | - | Dr. D. Prescott Mowry |
| 23. | Toronto | - | Dr. R.G. Ellis |

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-SECOND MEETING
OF THE
ASSOCIATE COMMITTEE
ON DENTAL RESEARCH

OTTAWA

MARCH 5-6, 1956

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- Appendix R Orthodontic Research.
- Appendix S History of Project DR-22.
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Initial Distribution.

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Twenty-second Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Council Chamber, National Research Council,

March 5 - 6, 1956

The twenty-second meeting of the Associate Committee on Dental Research was convened at 9:30 a.m., March 5th.

1. Attendance

Dr. J.B. Macdonald, Chairman
Dr. J.G. Aldous
Dr. H.J. Barrie
Dr. J.M.R. Beveridge
Dr. A.C. Burton
Dr. D.H. Copp
Dr. A.W. Ham
Dr. C.H. Husband
Dr. L.A. Kilburn
Dr. J.P. Lussier
Dr. E.G.D. Murray
Dr. E. Page
Dr. A.G. Racey
Brig. E.M. Wansbrough
Dr. C.H.M. Williams

Dr. J.B. Marshall, Secretary
Miss D.J. Wright

By invitation

Dr. C.P. Leblond
Dr. G. Nikiforuk
Dr. K.J. Paynter

Apologies for their absence were received from:

Dr. J.S. Bagnall
Dr. H.K. Brown

2. Chairman's Remarks

The CHAIRMAN opened the meeting by welcoming the guests of the Committee, Dr. G. Nikiforuk and Dr. K.J. Paynter, and noted that Dr. C.P. Leblond would attend the second session. He mentioned the change in status of two members of the Committee: Dr. Pagé who had left Laval University to become head of the Institute of Biology at the University of Montreal, and Dr. Murray, now research professor in the Department of Medical Research at the University of Western Ontario.

The CHAIRMAN told the Committee that in connection with the proposed establishment of a Dental School at the University of British Columbia, he had been asked to undertake, during the past year, a survey of dental education in Canada. His findings indicated that there had been no change in facilities in the past thirty years: in 1926 Canada had 5 dental schools, graduating 175 dentists per year; in 1956 there were still only 5 schools and there had been no increase in the annual number of graduates. In contrast to this, the number of medical schools in Canada had increased from 7 to 12 during the same period. In view of the large increase in population reported by the Gordon Commission, and the fact that the rate of retirement of dentists is now very high, the shortage will become more severe. Indications are that the profession is now serving only one-third of Canada's population. To remedy the shortage of qualified practitioners, it is expected that three or four additional dental schools will be opened in Canada within the next ten years, but at the present time the shortage of teachers and research workers is so acute that it will be difficult to staff such schools unless additional dental graduates can be persuaded to undertake post-graduate work with a view to following a full-time career of teaching and research.

A recent survey made by the Canadian Dental Association of research in Canada revealed how extremely limited the research effort is. Only two of the five dental schools have facilities for graduate training, and only two schools have on their staffs people trained in research. Practically all the dental research done in Canada has been sponsored by the Council's Associate Committee on Dental Research. In spite of the fact that there had been very little increase in the quantity of dental research in Canada of late, there has been a gratifying improvement in the quality, chiefly because basic science departments are now becoming interested in problems related to dentistry. At the present time very few dentists have the basic training required for research, and a longer training period would therefore be desirable. The Chairman felt that it was important for the

Committee to make every effort to stimulate and encourage research and research training, possibly through an expanded fellowship programme.

It was observed that dental schools appear to hold the shortsighted view that dental students do not need much training in the basic sciences. Dr. HAM pointed out that basic science courses are usually taken in the medical school where problems peculiar to dentistry were apt to receive little attention; the medical faculties were not always large enough to support these additional students and since the dental faculties usually failed to take any administrative responsibility towards them, the basic science departments did not take a particularly keen interest in teaching dental students. Dr. COPP suggested that Dental Schools might be responsible for the employment of one person for each basic science department. In this way, dental students would be assured of specialized training in their field and greater interest in research might be aroused. Dr. MURRAY said that he had attempted to have a lecturer in dental bacteriology and immunology appointed to the Dental Faculty at McGill, but without success. He felt that until the universities realize their responsibility in providing adequate specialized research training and facilities for dental students, there is little the Council can do to promote the special training required for dental research. He also pointed out that it was difficult to keep a dental student at school for graduate work in view of the considerably higher remuneration of practice, and unless there were obvious opportunities in the universities for a worthwhile career in dental research, men could not be blamed for ignoring the possibilities.

Dr. ALDOUS suggested that lack of interest in research might stem simply from a lack of knowledge as to what is being done in research. If teachers are not carrying on research - and in some dental schools research is still regarded as an unnecessary frill - students are not aware of problems which might be investigated. Dr. COPP felt that if interest in certain problems were promoted by the pre-clinical departments, the research could be carried on in the applied departments of dentistry, but here again the need for competent research teachers became obvious.

The CHAIRMAN reported that the Canadian Dental Association has endeavoured to promote interest in research by offering studentships to dental graduates; one of the stipulations of the awards, however, is that the successful applicant be assured of a position in a dental school after his training had been completed. No Dean of Dentistry had yet

been willing to give such assurance at the beginning of a graduate's research career. This was felt to be understandable but the Committee agreed that competent persons with research training would be assured of a position in a Canadian dental school.

3. Minutes of the Twenty-first Meeting

It was MOVED by Dr. Copp and SECONDED by Dr. Murray, THAT the minutes of the twenty-first meeting be taken as read and approved.

CARRIED.

4. Business Arising from the Minutes

(1) Referee's report on project DR-13 (Min. 8 (1), 21st Mtg.)

The CHAIRMAN read the report submitted by the referee who had been asked to make a recommendation as to the advisability of having a statistical analysis made of Dr. Smith's results. In view of the apparent unreliability of her data, a statistical analysis was not considered feasible; consequently, the funds encumbered for such an analysis had not been released during 1955-56, and no application for a grant for the coming year had been received.

It was the opinion of the Committee that the problem was worthwhile but it involved difficult organic analyses for which the grantee and her technicians might not have been qualified. Dr. COPP stated that Dr. Hodge at Rochester had done considerable work on fluoride determination and suggested that he be asked if there was a good, yet simple, method for the determination of fluoride which could be learned in a short time. If so, the method might be brought to Dr. Smith's attention, since the Committee felt that Dr. Smith's interest in matters relating to dental science was commendable and should be encouraged. Dr. NIKIFORUK reported that Armstrong had developed a micro method for the determination of fluoride in teeth similar to the Conway method for the determination of ammonia.

The availability of teeth for analysis was of some concern and it was hoped that Dr. Brown, of the Department of National Health and Welfare, who had been supplying Dr. Smith with teeth for her project, was still saving teeth for analysis in the event that the project was continued.

It was AGREED that Dr. Brown be asked to continue saving teeth for analysis, and that additional information be obtained from Dr. Hodge as to the feasibility of training people to carry out the necessary analyses. Dr. COPP agreed to get in touch with Dr. Hodge.

(ii) Executive action on applications from Dr. Kirkwood and Dr. Burgen (Min. 8 (ii), 21st Mtg.)

The CHAIRMAN reported that further information as to their proposed projects had been provided by the applicants at the Secretary's request, and the funds encumbered for these grants had therefore been released by the Executive.

(iii) Workmen's Compensation and pension payments (Min. 8 (i), 21st Mtg.)

The SECRETARY reported that Council held the opinion that the conditions of employment of technicians employed under grants should be similar to those of other technicians at the universities. Workmen's compensation on behalf of technicians employed under grants can therefore be regarded as a legitimate charge against a grant if a university normally adheres to the Workmen's Compensation Act. Similarly, the employer's contribution to a university pension fund can also be charged to a grant. It was emphasized, however, that by accepting such charges, the Council cannot be held in any way responsible for the persons on whose behalf such charges are paid.

(iv) Application form for grants in aid of research (Min. 9, 21st Mtg.)

The CHAIRMAN reported that no revision of the form used in applying for grants had been made during the past year. The SECRETARY stated that in view of the proposed modifications in the grant programme, with the introduction of term and continuing grants, action on this matter had been held in abeyance. He expressed the hope that a single grant application form can be developed for use in applying for all types of grants offered by the Council. The SECRETARY suggested that a method of application similar to that of the National Science Foundation in the United States might be worthy of consideration in this respect; their applicants are not required to complete a formal application, but are asked to outline the proposed research project for which they wish support. Some doubt was expressed, however, that such "proposals" would contain all the information required by the Council Committee before reaching a decision. Dr. HAM said that the National Cancer Institute had put considerable effort

into the development of a suitable application form for use by its applicants, and emphasized the fact that a prospective grantee should give sufficient information regarding his research to enable an outside referee who might not be fully acquainted with the applicant's work to evaluate its probable worth.

Following further discussion, it was MOVED by Dr. Ham and SECONDED by Dr. Husband,

THAT question 5 of the present application form be eliminated and incorporated in a supplementary sheet, that question 6 be eliminated, that applications for renewal of an existing grant be accompanied by an adequate progress report on the past year's work as well as an outline of the next year's programme, and that a note be included on the form to the effect that applicants should bear in mind the fact that the referees might not be entirely familiar with their work and adequate information should therefore be provided to permit a fair assessment of the research.

Prior to a vote on the motion, there was further discussion, as a result of which an amendment was PROPOSED by Dr. Copp and SECONDED by Dr. Kilburn,

THAT Dr. Macdonald, Dr. Ham and Dr. Marshall prepare a revision of the application form along the lines suggested by the Committee and take whatever action is required to establish its use.

CARRIED.

5. Report on Orthodontic Research

The CHAIRMAN introduced Dr. K.J. Paynter, Chairman of the Division of Post-graduate Studies in the Faculty of Dentistry of the University of Toronto, who had been invited to give a report on orthodontic research in Canada. Following an outline of the types of malocclusion, Dr. Paynter described the research methods used in orthodontic studies, the sources of material used in studies supported by the Committee, the organization of the Department of Orthodontics at the University of Toronto, and gave an appraisal of the future possibilities of orthodontic research. A summary of Dr. Paynter's report appears in APPENDIX R.

Following Dr. Paynter's report, Dr. HAM said that the Committee was indebted to Dr. Paynter for a clear exposition of a subject with which very few of the members were familiar. There was some discussion of the work being done at the Burlington Centre, which Dr. Paynter felt was of considerable importance, and Dr. HAM suggested that a well-trained anatomist might be of assistance in connection with the project in making measurements on the basis of the data obtained.

The CHAIRMAN thanked Dr. Paynter for attending the meeting, and noted that no applications have been received in connection with orthodontic projects for the coming year; this was attributed to the reorganization of the orthodontic training programme at the University of Toronto, which had been referred to by Dr. Paynter.

6. Consideration of Reports from Grantees

- (i) DR-1: Dr. J.J. Rae
Reported by Dr. Aldous

"Chemical mechanisms in dental caries."

A summary and progress report appear in APPENDIX C.

Following presentation of the report, Dr. Aldous expressed some doubt as to the ultimate aim of the project; he felt that the material being investigated did not seem to be sufficiently potent to warrant great concern. It was felt that insufficient information was provided to permit the Committee to evaluate the work being done, and Dr. BEVERIDGE suggested that a measurement of the bactericidal effect of the substance might have been attempted. Dr. HAM felt that work on the project seemed to be rather desultory and it should either be pursued more vigorously or dropped entirely.

- (ii) DR-22: Dr. C.H. Best (Dr. W.J. Linghorne)
Reported by Dr. Barrie

"An investigation of the mechanism of repair of the supporting structures of the teeth."

A summary and progress report appear in APPENDIX D.

Dr. Barrie described the project in considerable detail, basing his observations on his review of the grantee's report and on personal discussions with Dr. Linghorne. In order to

determine the role of periosteum in the regeneration of teeth and bones, Dr. Linghorne is carrying out experiments on the fibula of the dog. Dr. Barrie reported that the project was showing interesting results, but pointed out that at the present time the grantee bases his conclusions only on the developments in one experimental animal, and the deductions made should therefore be corroborated by further experiments. He suggested that a wider range of time intervals might be employed before any definite inferences are drawn as to the mechanism of repair of the fibula. Dr. Barrie expressed the opinion that the work was very important but that Dr. Linghorne needs some help in planning further experiments in connection with the project.

(iii) DR-23: Dr. C.P. Leblond
Reported by himself

"Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements."

A summary and progress report appear in APPENDIX F.

Dr. Leblond described, with the aid of slides, the study which he is making of the growth of the tooth, as revealed by radioautography. Following presentation of the report, Dr. COPP congratulated Dr. Leblond on an excellent piece of fundamental work on the nature of the dentin matrix and the formation and calcification of teeth.

Following some discussion of Dr. Leblond's methods, the CHAIRMAN thanked him for attending the meeting and presenting his report.

(iv) DR-28: Dr. O.J. Walker
Reported by Dr. Husband

"Methods for destroying the organic matter in teeth, bones, etc. prior to determination of fluorine."

A summary and progress report appear in APPENDIX O.

Dr. Husband described in further detail the work done by Dr. Walker during the past year. He reported that he had drawn to Dr. Walker's attention the method developed by Armstrong, as noted at the last meeting of the Committee. Unlike Armstrong's method, Dr. Walker's method requires ashing, which leaves a soluble residue with which to work.

Since Dr. Walker has not made application for a grant for 1956-57, this project is now to be considered complete. To the Chairman's question regarding publication of the results, Dr. Husband replied that some of the results are to be incorporated in a thesis.

(v) DR-37: Dr. D.H. Jenkins
Reported by Dr. Paynter

"Continuing studies in electromyographic techniques."

A summary and progress report appear in APPENDIX N.

During the course of his assessment of orthodontic research, Dr. Paynter touched on the work reported by Dr. Jenkins. He said that it had been found that there was no correlation between the temporal muscle and malocclusion. He felt that the cephalometric results obtained by Dr. Jenkins' group are very promising, but that a knowledgeable and qualified supervisor would help considerably in this phase of the work. He reported that Dr. Jenkins was getting good results from the use of a new monobloc appliance in correcting an Angle Class II type of malocclusion. Results on the past year's work are now being assembled for thesis publication, and no further grant has been requested for the coming year.

(vi) DR-43: Dr. S.G. Davis and Dr. H.R. MacLean
Reported by Dr. Husband

"The electropotentials of dental alloys."

The report requested by the Secretary in accordance with Minute 5 (vii) of the 21st meeting appears in APPENDIX P.

Dr. Husband said that Dr. Davis and Dr. MacLean had both been on sabbatical leave during the past year and no work had been done on the project since May of 1955. The Chairman noted that no application for a grant has been made for the coming year, so the present report may be regarded as final.

Dr. BURTON suggested that potassium chloride should have been used in the saline bridge in the cell, instead of the Krebs-Ringer solution reported by the grantees. He also commented on the fact that the grantees appear to regard the E.M.F.s of amalgamated alloys as much more predictable than they really are.

- (vii) DR-51: Dr. R.L. de C.H. Saunders
Reported by Dr. Ham

"Study of human dental pulp vessels by x-ray microarteriographic methods."

A summary and progress report appear in APPENDIX E.

Dr. Ham described the method used by Dr. Saunders in studying the vascular pattern of dental pulp and, after commenting on the excellent results obtained by microradiography, said that he felt that Dr. Saunders must now know everything there is to learn by this method about the blood vessels of the pulp. Dr. ALDOUS said that during the past summer, Dr. Saunders had made a trip to England and had spent considerable time in commercial and university laboratories in order to gain a thorough knowledge of his equipment and the techniques of microradiography; much of his time since returning to Dalhousie has been spent in getting his new equipment set up for use, and he now hopes to study the development of the vascular patterns from the foetal stage to the adult stage. Dr. HAM questioned the value of further elaboration of the topic, and suggested that a study of the blood vessels under various pathological and therapeutic conditions might be more useful.

- (viii) DR-52: Dr. L.F. Belanger
Reported by Dr. Burton

"Mechanism of bone and tooth formation in the light of autoradiography and histochemistry."

A summary and progress report appear in APPENDIX B.

Following presentation of Dr. Belanger's report, Dr. Burton said that the grantee appears to have tackled a lot of problems in an exploratory way and expressed astonishment at the number of elements which he had found time to study. He suggested that Dr. Belanger's reports would be more informative if in future he confined himself to the aspects of his work directly related to the dental side of the problem, rather than attempt to outline all the work being done in his laboratory.

After further discussion of the report, the CHAIRMAN suggested that Dr. Belanger be invited to attend the next meeting of the Committee to report on his investigations.

(ix) DR-55: Dr. M.R. Culbert
Reported by Dr. Paynter

"A study of maxillary growth as related to statural growth."

A brief report appears in APPENDIX M.

In accordance with Minute 5 (xii) of the 21st meeting of the Committee, the SECRETARY had requested a full report from Dr. Culbert, although he had not requested a grant for 1955-56. Due to the exigencies of practice and the building of new offices, however, Dr. Culbert had not been able to work on his project for some time. Dr. Paynter was of the opinion that no sustained research programme could be expected of practitioners such as Dr. Culbert and suggested that the Committee take this into consideration when considering future applications from part-time researchers.

(x) DR-56: Dr. D.J.E. Mitchell
Reported by Dr. Paynter

"A comparative study of palatal height, width and length in ancient and modern crania."

Following the last meeting (Min. 5 (xiii)), Dr. Mitchell was asked to send in a full report, although no grant was made for 1955-56. This report appears in APPENDIX L. Dr. Paynter felt that Dr. Mitchell, like Dr. Culbert, was probably too fully occupied with his work at the Hospital for Sick Children in Toronto and his practice in Peterborough to have much time to devote to research.

(xi) DR-57: Dr. G. Nikiforuk
Reported by himself

"The amino acid and carbohydrate composition of enamel using paper chromatography."

A summary and progress report appear in APPENDIX H.

Dr. Nikiforuk described, with the aid of slides, the work he had done in continuation of his project; the study has been extended to include the carbohydrate components of enamel as well as the amino acids. Following presentation of the report and some discussion as to the technique used, Dr. BEVERIDGE said that Dr. Nikiforuk was to be congratulated on a very nice piece of work in a very difficult field.

The CHAIRMAN thanked Dr. Nikiforuk for attending the meeting to present his report to the members of the Committee.

- (xii) DR-58: Dr. J.G. Aldous
Reported by himself

"The metabolism of procaine and related compounds."

A summary and progress report appear in APPENDIX A.

Dr. Aldous described in some detail the work he was doing on the toxicity and metabolic characteristics of local anesthetics. Using rabbits of the same sex and type, tests were made on convulsing time, pre-convulsing blood levels - as an indication of the diffusability of the drug injected - and the amenability of the drug to hydrolysis. Dr. Aldous felt that the claims made for the efficacy of local anesthetics were apt to be misleading, since they were based on subcutaneous toxicities, rather than on intravenous toxicities where the time element is such an important factor.

To Dr. BURTON's question as to the value of intravenous toxicity data to dental practitioners who make chiefly subcutaneous injections, Dr. HUSBAND replied that an intravenous assessment of a drug is of considerable importance since subcutaneous injections into such a highly vascular site as the mouth might inadvertently become intravenous.

- (xiii) DR-59: Dr. D.H. Copp
Reported by himself

"Effect of phosphorus deficiency on bones and teeth."

A summary and progress report appear in APPENDIX J.

Dr. Copp described his study of the effect of feeding rats on a diet very low in phosphorus. He showed slides to illustrate the severe demineralization of the skeleton resulting from five weeks on the diet, and the relative lack of effect of the diet on the teeth. He concluded that the growing soft tissues and teeth absorb most of the available mineral, at the expense of the bones.

- (xiv) DR-60: Dr. F.C. Fraser
Reported by Dr. Page

"Pathogenesis of cortisone-induced cleft palate in mice."

A summary and progress report appear in APPENDIX G.

Following presentation of the report, Dr. Pagé expressed the opinion that the work had been described well and in sufficient detail. The relation of Dr. Fraser's investigation to dental science was discussed following a question as to the possible application to the development of cleft palate in humans of the inferences to be drawn from the results with mice. Dr. HAM pointed out that cortisone did not induce cleft palate in rats, and suggested that monkeys might be more useful as experimental animals since they are more nearly like humans than mice are. It was the general opinion of the Committee that while a study of cleft palate is in part related to dentistry, Dr. Fraser's investigation appears to be mainly genetic in character.

(xv) DR-61: Dr. A.S.V. Burgen
Reported by Dr. Copp

"Secretion of protein in saliva."

A summary and progress report appear in APPENDIX I.

Dr. Copp felt that the report was not sufficiently detailed to give a clear picture of the work, but it was evident that techniques and methods had been worked out. Dr. BURTON suggested that it would be useful to do enzyme studies of salivary secretion in order to determine what the protein components of saliva are.

It was AGREED that Dr. Burgen be asked to report in person on his work at the next meeting of the Committee.

(xvi) DR-62: Dr. S. Kirkwood
Reported by Dr. Beveridge

"Role of the salivary glands in the extra-thyroidal metabolism of the thyroid hormone."

A summary and progress report appear in APPENDIX K.

Dr. Beveridge said the work was very interesting. He did feel, however, that Dr. Kirkwood had misinterpreted some of the statements of Chaikoff to which he referred. The Committee felt that the investigation deserved further support; however, no further funds have been requested since Dr. Kirkwood is going to Minnesota.

7. Report from Fellowship Holder

Dr. Lussier presented the report on hyaluronidase activity in oral bacteria submitted by Dr. C. Reynolds in

fulfilment of the requirements of the fellowship held in the University of Toronto during 1954-55 under the supervision of Dr. Bruce Crocker. The report was well received by the Committee. Dr. Reynolds is now reported to be working on a colorimetric method of determining hyaluronidase activity in saliva.

8. Review of Status of Project DR-22

At its 192nd Meeting, Council questioned the Committee's continuing support of project DR-22, a grant made to Dr. Best under which Dr. Linghorne was employed on a half-time basis. This grant is somewhat irregular in that the person employed under the grant, rather than the grantee, appears to be responsible for the project; this has been a matter of concern to the Committee for several years, as is evident from the outline of the project's history which appears in APPENDIX S.

The Committee gave further consideration to the way in which this grant might be regularized under some aspect of the Council's awards programme. Both the referee of the project and the Executive felt that the work should be supported; as Dr. BEVERIDGE pointed out, the problem of the mechanism of repair of supporting structures of the teeth is of vital interest in dentistry, and this investigation is the only one being carried out on the subject in Canada. Dr. WILLIAMS said that Dr. Linghorne is a very hard worker who conscientiously devotes all his afternoons to the research, but it was agreed that while he has excellent advisers around him he requires more assistance from workers in the dental field in planning his work.

The Executive was of the opinion that a small committee on periodontal diseases should be set up in Toronto to direct the work in this field. Such a group could assist Dr. Linghorne in re-orienting the problem and determining the direction his investigation might most profitably take next.

It was MOVED by Dr. Beveridge and SECONDED by Dr. Copp, THAT Dr. Barrie, Dr. Ham and Dr. Williams constitute a sub-committee on periodontal disease and assist Dr. Linghorne in the planning of his future work.

CARRIED

The CHAIRMAN said that the Council's concern was probably at least partly due to the fact that one research worker was being partially supported under a grant for a prolonged period of time. Dr. HAM pointed out that while Dr. Linghorne should not be allowed to become dependent in any

way on the income he derived from the grant, it was unlikely that this would happen since he could undoubtedly earn much more by giving up the research and devoting full time to his practice. Dr. BURTON agreed that a dangerous precedent might be set unless the university would employ Dr. Linghorne instead of giving him only an honorary appointment to enable him to carry on his research. He pointed out that this situation had been allowed to develop in the United States and as a result the universities are becoming precarious because professors are becoming increasingly dependent on grants for their income. In view of the Committee's strong reluctance to allow the project to lapse, he recommended that the grant now being made to Dr. Best be transferred to Dr. Linghorne and the payment of any income to Dr. Linghorne be stopped. Dr. WILLIAMS said that this would be a definite hardship to Dr. Linghorne in view of the high overhead of part-time dental practice; he felt that since the number of dental research workers in Canada is so small, Dr. Linghorne's interest in research should not penalize him financially. Dr. MURRAY pointed out that grants were made by Council in aid of research, not to support individuals directing research.

It was suggested that a half-time fellowship might be recommended for Dr. Linghorne, but Dr. MARSHALL did not feel that Council would approve such a recommendation.

It was finally decided that though the Committee definitely wishes the project to continue, it could not see its way clear to continuing its contribution to the personal income of Dr. Linghorne; this decision was to be borne in mind in reviewing Dr. Best's application for a grant for the coming year.

9. Applications for Grants:

The CHAIRMAN reported that the Executive had given very careful consideration to all applications at its meeting on the evening of March 5th, and its recommendations were presented to the meeting for discussion.

(1) Renewals

DR-1: Dr. J.J. Rae Amount Requested: \$1,080.

- (A) A bactericidal material in tooth enamel
- (B) Chemistry of calcification.

It was felt that little progress was being made on the first part of the project at the present time, and that the second part of the proposed project could not be profitably pursued by the methods proposed. The application was therefore NOT APPROVED.

DR-22: Dr. C.H. Best Amount requested: \$4,975.

"An investigation of the mechanism of repair of the supporting structures of the teeth."

It was MOVED by Dr. Barrie and SECONDED by Dr. Aldous, THAT the application be approved, including an amount of \$1,000 only for the salary of Dr. Linghorne for six months; that Dr. Best be advised that this is to be a terminal grant to him for this project, but that applications from Dr. Linghorne will be welcomed.

CARRIED

The amount approved for this project was therefore \$3,975.

It was the feeling of the Committee that Dr. Linghorne should be reassured as to the Committee's high opinion of his work on the project, in spite of its inability to continue paying him a salary under the grant; it was AGREED that Dr. Ham and the Chairman would visit him on their return to Toronto.

DR-23: Dr. C.P. Leblond Amount requested: \$3,300.

"Entry of carbohydrates into teeth as shown with the help of radioactive elements."

A grant of \$3,300 was APPROVED.

DR-51: Dr. R.L. de C.H. Saunders

Amount requested: \$2,130.

"Study of developing and adult human intra-dental and peridental vascular patterns by microradiographic methods."

The request for freeze drying equipment and accessories was not approved. Amount APPROVED: \$1,380.

DR-52: Dr. L.F. Belanger Amount requested: \$2,465.

"Mechanism of bone and tooth formation."

A grant of \$2,465 was APPROVED

DR-57: Dr. G. Nikiforuk Amount requested: \$2,980.

"Organic components of dental tissues."

A grant of \$2,980 was APPROVED

DR-58: Dr. J.G. Aldous Amount requested: \$3,000.

"The metabolism of procaine and related compounds. III and IV."

A grant of \$3,000 was APPROVED

DR-59: Dr. D.H. Copp Amount requested: \$5,450.

"Phosphorus deficiency effects on bones and teeth."

A grant of \$5,000 was APPROVED

DR-60: Dr. F.C. Fraser Amount requested: \$1,588.

"Studies on the pathogenesis of cleft palate."

A grant of \$1,588 was APPROVED. Dr. Fraser is to be advised that any future applications to the Council for support of this project should be directed to the Assisted Researches Committee, because the genetic nature of the problem removes it from the particular field of interest of the Associate Committee on Dental Research.

DR-61: Dr. A.S.V. Burgen Amount requested: \$5,950.

"Secretion of protein in saliva."

A grant of \$3,400 was APPROVED. It was felt that Dr. Burgen might have given a more detailed outline of his proposed research.

(ii) New Applications

Dr. K.J. Paynter Amount requested: \$3,000.

"Investigations into the nature of cementum."

A grant of \$3,000 was APPROVED

Dr. K.J. Paynter Amount requested: \$1,455.

"A histological study of teeth and periodontal tissues of guinea pigs reared on subminimal allowances of ascorbic acid."

A grant of \$1,455 was APPROVED

Dr. K.I. Melville

Amount requested: \$5,600.

"Studies on the mechanism of gingival changes associated with drug administration."

The Committee wished to support this application in order to encourage Dr. Melville's assistant, Dr. Francis, to do dental research, but were somewhat dubious as to the merits of the proposed problem. In view of its histological nature, the Committee questioned whether suitable facilities would be available in the Department of Pharmacology. It was also felt that the nature of the biochemical studies to be undertaken had not been sufficiently well explained. It was MOVED by Dr. Copp and SECONDED by Dr. Page,

THAT the amount of \$3,000 be encumbered, to be released by the Executive if a more satisfactory proposal is submitted.

CARRIED

Dr. G. Pelletier and Dr. J.G. Perreault

Amount requested: \$2,600.

"An evaluation of the disturbance of the dentine formation in the rat incisor following trauma of various origins."

A grant of \$2,600 was APPROVED. It was noted that this was the first application for a grant in aid of research to be received from the University of Montreal.

(iii) Summary

Applications were received for support for 14 projects amounting to \$45,573. The Committee approved 12 grants amounting to \$34,143 and encumbered an additional \$3,000 in connection with a further project.

10. Applications for Fellowships

(i) Renewals

(a) Dr. E.M. Madlener, D.D.S.

For the past two years, Dr. Madlener has held Graduate Dental Research Fellowships under the direction of Dr. J.B. Macdonald at the University of Toronto. He has now applied for a Senior Dental Research Fellowship in the amount of \$4,000 for 1956-57. The applicant is a careful, hard worker

who has had good academic training and is well qualified in bacteriology and biochemistry. He expects to get his Master's degree shortly and then to undertake a bacteriological study of anaerobic streptococci. A Senior Dental Research Fellowship, under the direction of Dr. J.B. Macdonald in the Division of Dental Research at the University of Toronto, was approved in the amount of \$4,000.

(b) Dr. M. Weiss, B.Sc., D.D.S.

Dr. Weiss has held a Graduate Dental Research Fellowship under Dr. Burgen in the Department of Physiology at McGill University for the past year. Dr. Burgen feels that he has made satisfactory progress during tenure of his Fellowship and recommends a renewal in the amount of \$4,000. A Graduate Dental Research Fellowship, under the direction of Dr. A.S.V. Burgen in the Department of Physiology at McGill University, was recommended in the amount of \$3,500.

(ii) New Applications

(a) Dr. Arthur Murray Hunt, D.D.S.

Dr. Hunt has applied for a Graduate Dental Research Fellowship. In addition to his D.D.S. degree, Dr. Hunt holds a Diploma in Dental Public Health from the University of Toronto and is now proceeding to the degree of M.Sc. in Dentistry. He was well recommended by Dr. Ham, Dr. McHenry and Dr. Paynter. A Graduate Dental Research Fellowship under the direction of Dr. K.J. Paynter in the Division of Dental Research at the University of Toronto was recommended in the amount of \$3,000.

(iii) Summary

Senior Dental Research Fellowship	(1)	\$4,000	
Graduate Dental Research Fellowships	(2)	<u>\$6,500</u>	\$10,500.

1. Action Taken by Executive Since Last Meeting

(i) Supplementary grants

The CHAIRMAN reported that applications for supplementary grants of \$198.00 had been received from Dr. G. Nikiforuk to permit the purchase of a pulverizing mill for use in connection with project DR-57, and of \$238.50 from Dr. L.F. Belanger to replace a paraffin oven used in his project DR-52. Letter ballots were sent to each member of the Executive and the applications had been unanimously approved. Supplementary grants in the amounts requested had therefore been made to Dr. Nikiforuk and Dr. Belanger.

(ii) Travel grants

The CHAIRMAN reported that the Executive had authorized, by letter ballot, travel grants of up to \$150.00 each for Dr. G. Nikiforuk and Dr. E.M. Madlener to enable them to attend the meeting of the International Association for Dental Research to be held in St. Louis, Missouri, in March of 1956. Both applicants were to present papers at the meeting.

12. Application for Travel Grant

The CHAIRMAN reported that Dr. J.J. Rae and his colleague, Mr. C.T. Clegg, had made application to the Committee for travel grants of \$100 each to permit them to attend the meeting of the International Association for Dental Research in March. In view of the fact that neither of the applicants was to present a paper at the meeting, and their project was no longer to be supported by the Committee, it was MOVED by Dr. Ham and SECONDED by Dr. Husband,

THAT the application be NOT APPROVED. CARRIED

It was noted that up to the present time it had been the Committee's policy to consider applications for travel grants in connection with attendance at meetings only from those who were to present papers at the meetings concerned. There was some discussion of the validity of this policy. Dr. HAM felt that the calibre of some meetings was degenerating because this ruling was also followed in many universities and as a result papers were sometimes presented not so much because they represented important contributions but because they made it possible for the author to claim for his travel expenses. It was generally felt that the decision as to the value of sending a man to a meeting should be based on the degree to which attendance at a meeting might benefit his research. A change in the Committee's policy was therefore recommended. It was MOVED by Dr. Copp and SECONDED by Dr. Burton,

THAT travel grants towards expense of attending meetings should be awarded on the merits of the case and the potential benefit to the research work of the applicant.

CARRIED

13. Stipends for Fellowships

Dr. WILLIAMS opened discussion on this topic by stating that in view of the salaries now being paid to other workers,

it is becoming increasingly difficult to attract graduates into research. He felt that an increase in the minimum stipend for Graduate Dental Research Fellowships from \$2,000 to \$3,000 would make them more attractive to students in the dental schools. It was noted that Council had approved a raising of the ceiling of Graduate Medical Research Fellowships to \$4,500 at its meeting in December 1955, but it was the consensus of opinion that only the establishment of a more reasonable initial stipend would make the Fellowships more attractive to new graduates. It was pointed out by Dr. BEVERIDGE that while people may not be encouraged to go into research by the amount of money offered, the lack of suitable remuneration may act as a definite deterrent. Dr. COPP felt that the stipends offered in connection with Fellowships should be increased substantially to meet the present cost of living, in order to prevent Fellows from actually going into debt. Dr. MURRAY pointed out that it was not necessary to give a successful applicant the minimum stipend for an initial Fellowship, but it was generally felt that if research supervisors could advise students that the Fellowships were valued at \$3,000 to \$4,000, depending on qualifications, more applications would be received. Following considerable discussion of the problem and of the need to encourage more students to take graduate work in order to build up qualified teaching and research staffs for the new dental schools which are expected to be established in the next ten years, it was MOVED by Dr. Williams and SECONDED by Dr. Beveridge,

THAT the Committee recommend to Council that the range of Graduate Dental Research Fellowships be changed from \$2,000 - \$3,500 to \$3,000 - \$4,000.

CARRIED

14. Finances

(i) Financial status of the Committee

The CHAIRMAN reported that the financial status of the Committee as at February 29th was as follows:

Allotment, 1955-56 \$40,700.00

Expenditures

Grants	\$30,786.50
Fellowships	6,000.00
Travel	568.02
Duplication	105.76
Reprints	5.41

\$37,465.69

	Forward	\$37,465.69	
Estimated additional expenses			
Cost of meeting	\$1,800.00		
Duplication	50.00	1,850.00	
			\$39,315.69
Estimated free balance		1,384.31	\$40,700.00

It was MOVED by Dr. Copp and SECONDED by Dr. Lussier THAT the Executive be authorized to recommend to Council the expenditure of up to \$1,200 of the estimated free balance for the present fiscal year for the purchase of items of equipment required by the grantees for the coming year, and reduce the grants for the coming year accordingly.

CARRIED

(ii) Estimates for 1957-58

The CHAIRMAN pointed out that no further meeting of the Committee would be held prior to the time when estimates for 1957-58 would be required. In view of the slightly larger stipends anticipated for Fellowships, it was felt that some increase in the budget of the Committee should be requested. It was therefore MOVED by Dr. Burton and SECONDED by Dr. Copp,

THAT the Associate Committee on Dental Research request an allotment of \$50,000 for the fiscal year 1957-58.

CARRIED

15. Membership of the Committee

The CHAIRMAN reported that Dr. Cline, whose appointment to the Committee was recommended to Council following the 21st Meeting, and subsequently approved, had declined to accept membership on the Committee. No replacement has therefore been made of Dr. R.H. McDougall. He also informed the Committee that the terms of appointment of Drs. Husband, Racey, Beveridge, Aldous and Copp would expire on March 31, 1956, but pointed out that they were all eligible for re-appointment to a second three-year term.

It was MOVED by Dr. Burton and SECONDED by Dr. Ham, THAT the five retiring members be recommended to Council for re-appointment for a further three-year period, to expire March 31, 1959.

CARRIED

In connection with the nomination of a successor to Dr. McDougall, the CHAIRMAN said that the new member should, in accordance with the terms of reference of the Committee, be a representative of the dental profession, and should preferably be from the West in order to maintain a fair geographical distribution. Following consideration of several names, it was MOVED by Dr. Husband and SECONDED by Dr. Copp,

THAT Dr. Robert J. O'Neill, a pedodontist in Vancouver, be recommended to Council for appointment to the Committee for a three-year term to expire March 31, 1959.

CARRIED.

16. Publications of Grantees

The SECRETARY reported that reprints of the following three papers had been provided to the Committee by grantees during the past year:

"Autoradiographic detection of radiosulfate incorporation by the growing enamel of rats and hamsters." by L.F. Belanger. J. Dental Res., 34: 20-27, 1955.

"Autoradiographic visualization of Ca^{45} intake by normal and pathological cartilage in vitro." by L.F. Belanger. Proc. Soc. Exper. Biol. & Med., 88: 150-152, 1955.

"Studies in the reattachment and regeneration of the supporting structures of the teeth. III. Regeneration in epithelized pockets." by W.J. Linghorne and D.C. O'Connell. J. Dental Res., 34: 164-177, 1955.

17. "Bibliography on Caries Research"

The SECRETARY reported that 6 copies of the bibliography had been sold since the last meeting, leaving a supply of 36 copies still on hand.

Dr. COPP drew the attention of the Committee to an excellent bibliography on bones and teeth, which was now available from the Superintendent of Documents, Washington 25, D.C. This volume covers the period from 1933 to 1954.

18. Publication of a "Canadian Journal of Dental Research"

The CHAIRMAN raised the question of the possible advantages to be gained by sponsoring a "Canadian Journal of Dental Research". He reported that the only existing journal

specifically designed for the publication of dental research papers was the "Journal of Dental Research", published by the International Association for Dental Research. At the present time, that Journal's backlog is so great that approximately 18 months now elapses between submission and publication of a paper. He was of the opinion that the establishment of another dental research journal would give added stimulus to researchers in the field.

Dr. BURTON inquired about the number of papers now being published by Canadians in this field. The Chairman said that he had not yet had an opportunity to investigate this point, and it was agreed that a minimum of between 40 and 50 papers per year would be required before a journal could justifiably be started.

It was suggested by Dr. HAM, and AGREED, that the Chairman should look further into this matter and obtain additional information for presentation at the next meeting.

19. Appreciation of Services

The CHAIRMAN drew the attention of the meeting to the fact that since Dr. Murray was retiring from the Council this year, he would not be attending future meetings of the Associate Committee on Dental Research. He thanked Dr. Murray for his contribution to the deliberations of the Committee during the past two years, and expressed the regret of the members that he would no longer represent Council on the Committee.

20. Date of next meeting

The date of the next meeting was left open, to be arranged by the Chairman and the Secretary for the Spring of 1957, when information is available as to the dates on which other Council Committee meetings are to be held.

The meeting adjourned at 2:45 p.m., March 6, 1956.

APPENDIX A

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: The metabolism of procaine and related compounds.
II. Diethyl aminoethyl-4-amino-2-propoxy benzoate hydrochloride (Ravocaine HCl).

Grantee: Dr. J. G. Aldous,
Dept. of Pharmacology, Dalhousie University

Project No.: DR-58 **Amount of Grant:** \$2,430.

SUMMARY OF WORK

A study of both the in vitro and in vivo metabolic characteristics of Ravocaine in the rabbit show it to be a compound about five times as toxic as Procaine, which diffuses quite readily across the blood-brain barrier, and which is only moderately amenable to enzymatic hydrolysis by whole blood.

after mixing. From these data the velocity constant for the hydrolytic reaction was calculated using the equation:

$$k = \log \frac{a}{x} \cdot \frac{1}{t}$$

where a is the concentration at zero time and x the concentration at time t . Procaine was found to be hydrolyzed very slowly by rabbit blood ($k = 0.007$) while Unacaine showed a rapid disappearance ($k = 0.036$). These data indicate that Unacaine is hydrolyzed 5 times faster than Procaine and suggest that the differences in s.c. toxicity might be accounted for on rates of destruction rather than rates of diffusion.

It is apparent from the foregoing that the in vitro hydrolysis of a local anesthetic, when taken in conjunction with the i.v. toxicity gives very useful information. It should be emphasized however, that i.v. toxicities have very little value unless they are expressed in terms of the time over which the injection is made. For this reason we have used a constant-speed injection machine to deliver solutions of varying concentration. From these data we can calculate in mg./kg/min. the maximum rate of injection which an animal can tolerate without showing signs of toxicity (convulsions). This rate is in reality an expression of the overall process of detoxification.

In the present study Ravocaine was chosen for investigation because its i.v. toxicity is quite different from that of Procaine and Unacaine.

Methods

Intravenous toxicity. Solutions of various strengths of Ravocaine were injected into the marginal ear vein of the rabbit via a polyethylene catheter, at 0.5 ml/min. until the animal exhibited typical convulsive reactions. A plot was then made of convulsing time vs. rate of administration and from this curve, the maximum non-toxic rate of administration was determined by extrapolation. The results are shown in Figure 1.

Pre-convulsing blood levels. Samples of the blood were withdrawn from the animal by cardiac puncture at several times during the injection, and before convulsions appeared. These were analyzed for Ravocaine. The data are shown in Table 1.

In vitro hydrolysis. A mixture of local anesthetic and heparinized blood, incubated at 37°C was sampled and analyzed at

various times after mixing.

Colorimetry. The method of Terp (1) was modified to allow the direct determination of Ravocaine. After diazotization, the color was developed with N-1-naphthylethylenediamine.

Intravenous toxicity. When the rate of administration was varied from 1.15 to 5 mg. per minute convulsions were observed at the times shown in Figure 1. When given at 1.1 mg. per minute, convulsions were never observed; thus the maximum non-toxic rate of administration was quite critical. Since the average weight of animals used in these experiments was 3.15 kg., the maximum non-toxic rate of administration based on body weight is 0.317 mg./kg/min. This value is to be compared with those of 1.5 for Procaine and 1.26 for Unacaine, and indicates, on this basis, that Ravocaine is about 5 times more toxic than Procaine. Information from the literature (2) shows almost the same quantitative relation when acute lethal i.v. doses are compared.

Pre-convulsing blood levels. The fact that the data of Figure 1 were obtained at about one-quarter the rates of administration that were used in the Procaine and Unacaine study, suggested that we might find the blood level of Ravocaine to be quite low.

The data of Table 1, showing the pre- and post-convulsion blood level at three different rates of administration, bears out this expectation and in addition shows that the pre-convulsing blood level does not rise markedly above that when convulsions are observed. This would suggest that in contrast to Procaine and Unacaine, Ravocaine diffuses quite readily across the blood-brain barrier.

TABLE 1

	Rate of administration mg./min.	Sampling time minutes	Ravocaine conc. µg./ml. blood.
A.	1.20	3.00	2.70
		7.80	4.84
		11.16	convulsed
		11.66	3.30
B.	1.5	2.75	2.12
		6.80	convulsed
		7.5	1.60
		15.25	1.00
C.	3.5	1.50	5.84
		2.33	6.48
		3.10	convulsed
		4.30	3.00
		7.10	2.75

In vitro hydrolysis of Ravocaine. The rate at which Ravocaine disappears from the blood when added to it in the test tube was observed to vary greatly. The data could be divided quite sharply into two classes: Four experiments showed a linear relation with time and a very slow rate of hydrolysis. These data resembled those obtained with Procaine. Six experiments showed a fairly rapid hydrolysis - the data fitting a hyperbolic curve, and resembling the results obtained with Unacaine. The reason for these two types of data is obscure at present.

The velocity constant of the average of all rates of hydrolysis over the first 5 minutes was calculated to be 0.032 which is a value intermediate between that of Procaine and Unacaine.

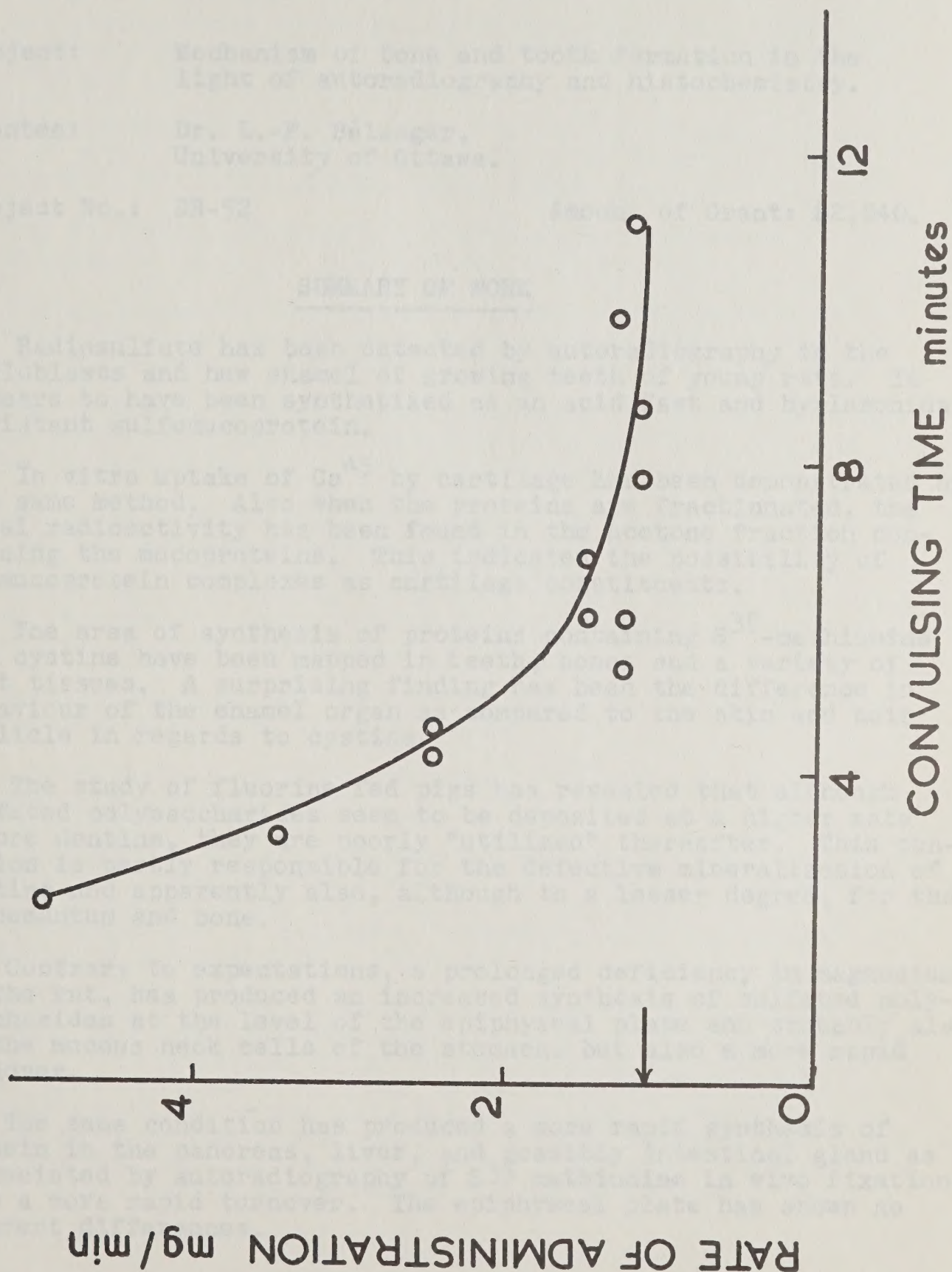
The results of the program of investigation so far seem to indicate that factors responsible for toxicity and for duration of anesthetic action may vary a great deal within this group of drugs. For example, Procaine and Unacaine with a similar i.v. toxicity, diffuse rather poorly. However, Unacaine is readily destroyed by enzyme action, Procaine is not. This raises the interesting possibility that the action of drugs of the Unacaine type might be greatly potentiated by the use of a cholinesterase inhibitor, whereas that of the Procaine type might better be prolonged, as it is at present, by a vasoconstrictor. It is hoped that this suggestion may be tested in the coming year.

Ravocaine differs most markedly from the other two drugs which have been studied in its rapid rate of diffusion and consequent low effective blood level. Because it is only moderately amenable to hydrolysis, the action of this compound might better be prolonged by vasoconstrictors. However, since all the local anesthetics are, to a varying degree, hydrolyzed by pseudocholinesterase, it seems worthwhile to test them all with additions of a cholinesterase inhibitor, as well as a vasoconstrictor, because in doing so one may be able to use much lower, but equi-effective concentrations of local anesthetic.

References

- (1) Terp, P. Acta Pharmacol. et Toxicol. 6: 269. 1950.
- (2) Luduena, F.P. and Hoppe, J.O. J. Pharmacol. Exper. Therap. 104: 40. 1952.

FIGURE 1



APPENDIX B

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Mechanism of bone and tooth formation in the light of autoradiography and histochemistry.

Grantee: Dr. L.-F. Bélanger,
University of Ottawa.

Project No.: DR-52

Amount of Grant: \$2,040.

SUMMARY OF WORK

1. Radiosulfate has been detected by autoradiography in the ameloblasts and new enamel of growing teeth of young rats. It appears to have been synthesized as an acid fast and hyaluronidase resistant sulfomucoprotein.
2. In vitro uptake of Ca^{45} by cartilage has been demonstrated by the same method. Also when the proteins are fractionated, the total radioactivity has been found in the acetone fraction containing the mucoproteins. This indicates the possibility of Ca-mucoprotein complexes as cartilage constituents.
3. The area of synthesis of proteins containing S^{35} -methionine and cystine have been mapped in teeth, bones and a variety of soft tissues. A surprising finding has been the difference in behaviour of the enamel organ as compared to the skin and hair follicle in regards to cystine.
4. The study of fluorine fed pigs has revealed that although sulfated polysaccharides seem to be deposited at a higher rate in pre dentine, they are poorly "utilized" thereafter. This condition is partly responsible for the defective mineralization of dentine and apparently also, although to a lesser degree, for that of cementum and bone.
5. Contrary to expectations, a prolonged deficiency in magnesium in the rat, has produced an increased synthesis of sulfated polysaccharides at the level of the epiphyseal plate and probably also in the mucous neck cells of the stomach, but also a more rapid turnover.
6. The same condition has produced a more rapid synthesis of protein in the pancreas, liver, and possibly intestinal gland as appreciated by autoradiography of S^{35} methionine in vivo fixation; also a more rapid turnover. The epiphyseal plate has shown no apparent differences.

PROGRESS REPORT

The following original articles dealing with the above-mentioned subject have been published in 1955:

Autoradiographic detection of radiosulfate incorporation by the growing enamel of rats and hamsters, by Leonard F. Bélanger. J.D.R. 34: 20-27. 1955.

Autoradiographic visualization of Ca^{45} intake by normal and pathological cartilage in vitro, by Leonard F. Bélanger. Proc. Soc. Exper. Biol. & Med. 88: 150-152. 1955.

The following article, whose summary was presented in the 1954 report, is in press with Anatomical Record:

Autoradiographic visualization of the entry and transit of S^{35} methionine and cystine in bones, teeth and various other tissues of the growing rat, by Leonard F. Bélanger.

The following article already approved for publication by the Oak Ridge Institute of Nuclear Studies and the U.S.A.E.C., will be submitted shortly to the American Journal of Anatomy:

The effects of fluorine feeding on the organic matrix of the hard tissues of growing pigs, by L.F. Bélanger, W.E. Lotz, W.J. Visek and C.L. Comar, Dept. of Histology and Embryology, University of Ottawa, Canada, and the University of Tennessee - Atomic Energy Commission Agricultural Research Program, Oak Ridge.

Further progress has been accomplished in the study of in vitro uptake of Ca^{45} as reported below.

Biochemical studies on interpretation of in vivo and in vitro radioisotopic uptake:

In vivo S^{35} in the rat stomach: see "Démonstration par électrophorèse et autoradiographie d'une sulfomucoprotéine synthétisée à l'aide de radiosulfate par la glande gastrique du rat", by M. Crevier and Léonard F. Bélanger. Comptes rendus des séances de la Société de Biologie, 148: 1530, 1954.

In vitro Ca^{45} : Fresh cartilage from the tibia and femur of young rats was carefully dissected, cut in small pieces and placed for 2 hrs. in a solution of $\text{Ca}^{45}\text{Cl}_2$ containing $\frac{1}{2}$ - 500 $\mu\text{c}/50 \text{ ml}$. carrier free Ca. The tissue was washed carefully with distilled water and then homogenized in a Potter grinder. Fractions were obtained as described above (paper by Crevier and Bélanger noted above) and prepared for electrophoresis. The total radioactivity of the initial sample was preserved in the mucoprotein fraction

but became lost each time when electrophoresis of this fraction was attempted.

Counts on Fractions

(80 μ l deposited on filter paper, dried and counted at 1 cm. from a 10 μ mica window G.M. tube)

Total extract 404 counts per min.

Trichloroacetic supernatant . . 375 " " "

Acetone fraction
(mucoprotein) 578 " " "

Acetone supernatant 135 " " "

Background on paper 140 " " "

It is of interest to note that when the paper strip was stained, 4 definite spots were recognized: 3 near the starting line and one at a considerable distance from these in a fashion similar to that found in the rat stomach. This highly mobile substance stained metachromatically with toluidine blue. In the acetone fraction, this was the only spot detected.

Conclusions: It is possible that the attempt to isolate by electrophoresis the molecules which "adsorb" the radio-calcium in vitro, has failed on account of the difference in the electric charge of Ca and that of the mucoprotein. When the current is applied, the complex must be dissociated and only the mucoprotein portion is mobilized towards the anode. On the other hand, the fact that the acetone fraction containing the mucoprotein is as much radioactive as the total extract while the acetone supernatant has a paper background count is strongly in favour of a Ca-mucoprotein complex existing up to the time of electrophoresis.

The study of sulfated polysaccharide synthesis and protein synthesis in normal and magnesium deficient rats has been initiated as reported below:

Young male rats of 40 gms were fed for periods of up to 30 days with a commercial casein diet containing less than 1 mg of magnesium per 100 gms of diet. In one experiment, the controls were fed on fox chow. A second group was fed the Mg deficient diet supplemented with 400 mgs Mg Cl₂ per 100 gms of diet. At the end of 30 days in this experiment, 9/14 of

the Mg deficient animals had survived. Six of these and six controls were injected with 5 $\mu\text{c/gm}$ $\text{H}_2\text{S}^{35}\text{O}_4$ and sacrificed in pairs at intervals of 4 hrs., 2 and 4 days. The tissues were prepared for integrated autoradiography and also for histological and histochemical staining.

Another group of 6 Mg deficient animals and 6 controls later in the year, were injected at 30 days with 3 $\mu\text{c/gm}$ DL-methionine S^{35} and sacrificed at intervals of 4 hrs., 4 and 8 days. The tissues were prepared as above.

Radiosulfate synthesis in Mg deficiency:

In view of the decreased growth rate and the histological appearance of the epiphyseal plate which has already been reported as atrophic (Dick and Prior '51), we expected to find a lower rate of sulfated polysaccharidesynthesis in the animals on Mg deficiency. It appears from this first series that the amount of radioactivity present in the epiphyseal plate 4 hrs. after the injection of tracer, is greater than that of controls as appreciated by the relative darkening of the photographic emulsion. Moreover, the ability to synthesize more radiosulfate seemed to extend in the deficient animals to the cartilage of the articular surface. The epiphyseal plate of animals sacrificed 2 and 4 days after the introduction of tracer has produced an autoradiograph which was definitely in favour of the controls. No such apparent differences were recognized in the soft tissues except maybe at the level of the mucous neck cells of the glandular stomach which appeared to have produced an autoradiograph stronger in the Mg deficient.

Protein synthesis in Mg deficiency:

Animals treated with methionine S^{35} have produced a weak autoradiograph at the epiphyseal plate when compared with those above. There was no apparent difference between the Mg deficient group and the controls. Weak reactions were also picked up at the growth areas of the bone (epiphyseal, periosteal, endosteal) and in the root portion of dentine. On account of technical difficulties in producing sections and comparable autoradiographs, no report can be presented at this stage, on the bones and teeth of animals sacrificed 4 and 8 days after the introduction of tracer. When the soft tissues were examined, several glands, the pancreas, (a picture is attached to the current application) the liver, the enzymic stomach and possibly also the gland of Lieberkühn appeared to have produced a stronger autoradiograph in the Mg deficient animals at 4 hrs. At 4 days, the record was much less intense and possibly in favour of the controls.

Special studies of the endocrine pancreas:

In the autoradiographs of both radiosulfate and radio-methionine, the island of Langerhans appeared as negative light spots particularly prominent in the second series against the intensely dark exocrine acini. It seemed at first that they were hypertrophic. In order to recognize a possible functional change, the glycemia was evaluated by micro dosage in one series of animals by our summer assistant, Mr. A. E. Gilbert, and Dr. Crevier. As revealed by the attached table, no difference was found in the glycemia of the Mg deficient and control animals.

Counts per area of sections revealed no difference in the number of islands.

Counts of islands which were above 1 cm. in diameter in a projected image of x 144 showed no difference either, indicating that not only was there no difference in the number of islands but also that the islands present were not larger in either group.

The effect of Mg deficiency on blood formation and destruction:

The stained sections of the present material have revealed another unreported manifestation of Mg deficiency. The spleen of the deficient animals was considerably enlarged in all cases. On microscopic examination, both red and white pulp appeared involved. The red pulp showed a great number of phagocytic elements replete with fragments in which organic iron was demonstrated (see pictures in current application). The nodules were hypertrophic and showed the presence of numerous young cells at the periphery. The bone marrow also appeared generally hypertrophic. In smears of peripheral blood made at weekly intervals from blood of the tail, young forms appeared in the last week of the experiment in at least two of the Mg deficient animals. They involved both the red and the granular series.

Conclusions: Surprising as it may seem, it appears that the synthesis of sulfated mucopolysaccharides and that of glandular proteins is stepped up in magnesium deficiency, while on the other hand the turnover appears faster. This, of course, requires confirmation (see current application).

There is no functional or anatomical involvement of the endocrine pancreas yet recognized.

There are definite haematologic changes in magnesium deficiency. These involve hyperplasia of the bone marrow

Blood Glucose Determination in Magnesium Deficient Rats and in Controls by the Photocolorimetric Method of

Somogyi - Nelson

Groups	Days of Experiment											
	5 days	9 days	12 days	16 days	19 days	24 days	27 days	30 days				
	mg% %	mg% %	mg% %	mg% %	mg% %	mg% %	mg% %	mg% %				
Mg def- icient	121 109	101 106	114 105	101 105	95 100	109 99	102 97	108 100				
Controls	110 -	95 -	108 -	102 -	95 -	110 -	105 -	108 -				

and of the white pulp of the spleen, the appearance of young forms in the blood stream and more rapid destruction of red cells and exaggerated phagocytosis in the red pulp of the spleen.

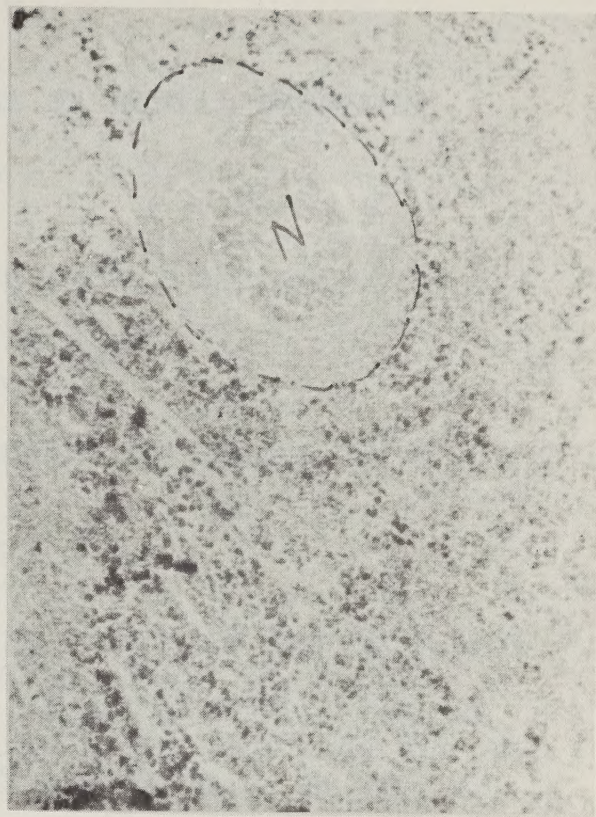
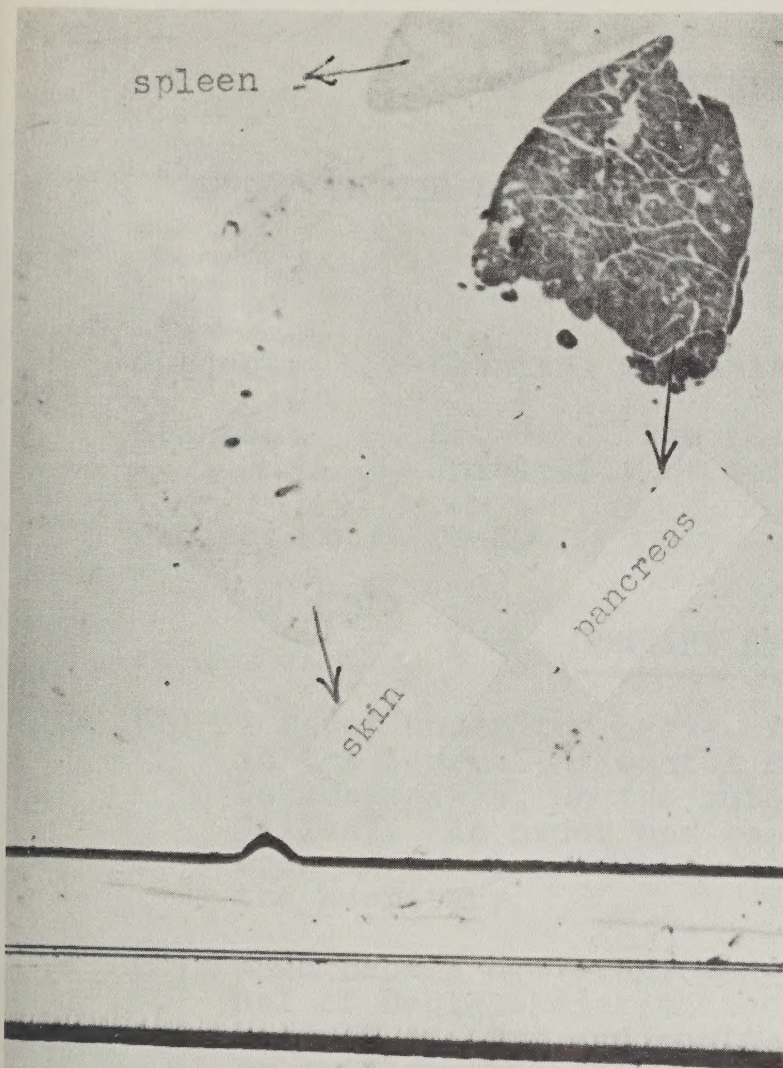
The following review article was published in 1955:

Formation of bones and teeth as visualized by radio-autography, by C.P. Leblond, L.F. Belanger and R.C. Greulich. Annals N.Y. Acad. Sci. 60: 629-659. 1955.

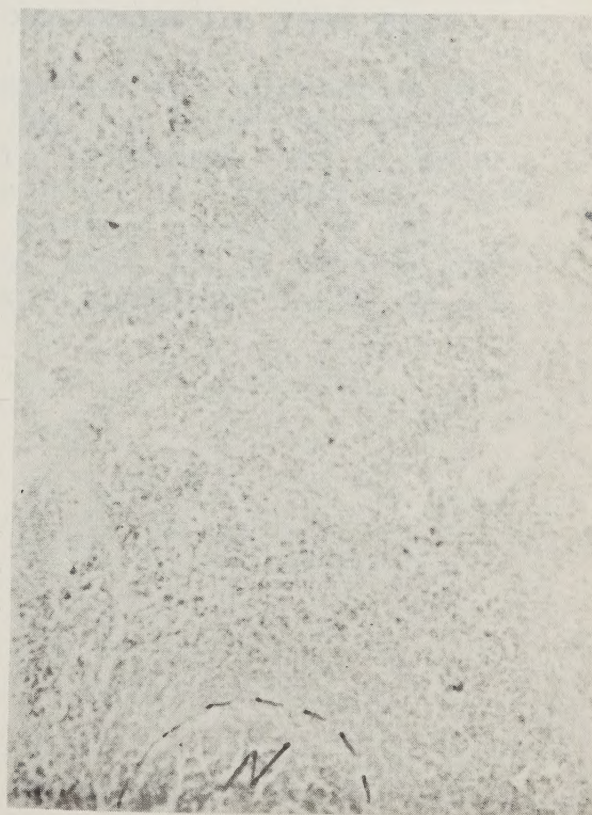
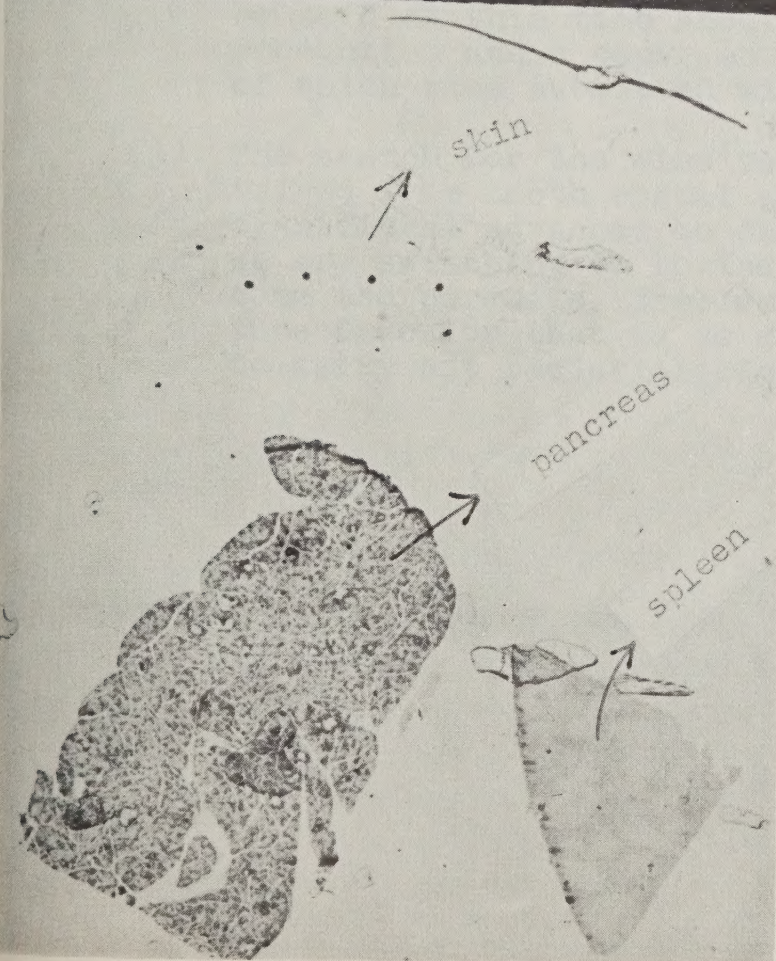
The following composite paper has been presented at the Ciba symposium (London) in July, 1955 and will be a chapter in the 1956 issue entitled "Bone structure and metabolism":

Autoradiographic studies of the formation of the organic matrix of cartilage, bone and the tissues of teeth, by Leonard F. Belanger.

Mg Def.



Mg Def. (Fig. 2)



Control (Fig. 3)

APPENDIX C

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Chemical mechanism in dental caries.

Grantee: Dr. J. J. Rae (C. T. Clegg)
University of Toronto.

Project No.: DR-1 Amount of Grant: \$540.

SUMMARY OF WORK

- (1) A paper under the aegis of this committee has appeared in the Journal of Dental Research, Vol. 34, p.886, December 1955, on the subject "Ammonia Production in Saliva". An order has been placed for reprints and as soon as these are available 25 copies will be sent to the secretary.
- (2) When papers are submitted for publication to The Journal of Dental Research there is a delay of 18 months between receipt and publication and so nothing more can be said at this time about a second paper on ammonia production and a paper on lactic acid production, both of which were submitted with our report a year ago.
- (3) The search for the elusive bactericidal substance obtained from tooth enamel goes on. A brief report on significant advances to date is attached. At present we are switching to bovine teeth in an attempt to overcome the perennial problem of ending up with a very pure fraction that is so minute that it is impossible to carry out bacteriological and chemical tests on it.

PROGRESS REPORTA Bactericidal Substance Towards Lactobacillus Acidophilus Prepared from Tooth Enamel.

Wakeman and Smith et al reported that the bacterial counts from unground teeth of the cotton rat are much lower than the corresponding counts from ground teeth. They concluded that the majority of the organisms are located in the deep fissures of the teeth. It might also be concluded that ground tooth substance would be a growth-promoting factor for lactobacillus. To test this assumption ground human tooth enamel (50 mg) prepared by the Manley and Hodge (2) technique was added to 4 ml of broth culture media and the media inoculated with lactobacillus. Surprisingly enough, complete inhibition of the growth of the bacteria was observed when 0.1 ml of the 24-hr culture was incubated for 4 days on an agar plate. The addition of the same amount of ground calcium phosphate to the broth culture tube showed no such inhibitory action on lactobacillus under the same conditions. Freshly ground whole tooth substance was found to have no inhibitory effect on lactobacillus growth. This led us to suspect that the bromoform used in the separation of dentine and enamel in the Manley and Hodge technique was causing the observed inhibition. However the dentine function of whole tooth substance did not cause inhibition. Ground bone meal or calcium phosphate when shaken with bromoform and dried, showed no inhibition. Bromoform itself (0.5 ml to 4 ml of broth culture) did not appreciably inhibit the growth of lactobacillus. 10 mg. of ground tooth enamel, separated from dentine by the bromoform density method, even after heating for 4 hours at 160° completely inhibited the growth. (The boiling point of bromoform is 149°.) These results led us to assume that bromoform when shaken with tooth enamel forms a complex which inhibits the growth of lactobacillus.

A solution of 10% bromine in chloroform could be substituted for the expensive bromoform without any essential alteration in the above results. Chloroform, however, does not produce any inhibiting substance when shaken with tooth enamel.

After the bromoform-treated tooth enamel is extracted with ethanol, acetone or methanol, the inactive solid residue can be made to yield more of the active material by again shaking it with bromoform.

The procedure for the production of inhibiting substance was as follows:-

Two hundred extracted human teeth were dried in an oven at 120° for 24 hours. The enamel, along with a considerable quantity of dentine, was cracked off with pliers, and the resulting mixture was ground in a pulverizer to between 60-100 mesh. Three lots of 20 gm each of the tooth substance were shaken with bromoform (or with 10% bromine in chloroform) for 1 hour. The mixture was filtered, and the solid residue was dried in an oven at 160° for 24 hours. The residue (tooth substance) was extracted with methanol in a Soxhlet apparatus for 4 hours. This extract, which we called Filtrate I, was found to be bactericidal to lactobacillus in concentrations as low as 0.1 mg of solids per ml. of broth culture. 200 ml. of Filtrate I was passed through a column of Al_2O_3 . The column was washed with methanol and then eluted with 50 ml. of N/10 HCl. Both the methanol filtrate and the N/10 HCl elutriate were tested against a 24 hour culture of l.b.a. It was found that the methanol filtrate no longer inhibited the growth of l.b.a. and the N/10 HCl elutriate inhibited l.b.a. growth in concentrations as low as 0.1 mg of solids per ml of broth culture. Qualitative tests on Filtrate I showed no bromide, nitrogen, phosphorus or sulfur. A positive test for the presence of chloride was obtained. The ashed residue from Filtrate I was found to give a positive test for ferric iron.

No bactericidal substance was obtained by shaking with bromoform tooth enamel which previously had been ashed at $900^{\circ}C$ for 4 hours.

X-ray diffraction is at present being used in an attempt to identify a crystalline material obtained from the Al_2O_3 column elutriate. (In one such test the material turned out to be calcium sulphate which was shown to be inactive.) In an attempt to increase the yield of the bactericidal material bovine teeth are now being used and the work on this substance is just getting started.

References:

- Wakeman and Smith et al. Jour. Dental Res. 27: 41, 1948.
Manley and Hodge, Jour. Dental Res. 18: 133, 1939.

APPENDIX D

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth.

Grantee: Dr. C. H. Best, (Dr. W. J. Linghorne)
University of Toronto.

Project No.: DR-22

Amount of Grant: \$4,200.00

SUMMARY OF WORK

Since the last report to the Committee, three papers have been written. One has been published in the Journal of the Canadian Dental Association. Through an error, reprints of this paper were not secured. Another, Study IV in the series of papers to the Journal of Dental Research, is in press. Reprints will be forwarded to the Committee when available. The third paper was written as a thesis for the Master of Science degree in dentistry. It is expected that this paper will be published.

Dr. Zanders, Professor of Periodontia, University of Minnesota, was a recent visitor to this department. It might be of interest to the Committee to know that he stated that the work on regeneration, as outlined in Studies I, II, and III is being repeated in several universities in the United States, using the monkey as the experimental animal. Dr. Zanders is repeating the work at the University of Minnesota. His findings so far indicate that the experimental epithelialized pockets produced by the method developed in our investigation are, from the histopathological point of view, quite comparable to periodontal pocket in humans.

Dr. S. C. Miller, Professor and Chairman of the Department of Periodontia and Oral Medicine, New York University, requested slides of sections from Study III to present before the International A.R.P.A. meeting in Venice this year. A number of slides from this study were sent to Dr. Miller for this purpose.

The four main methods of approach to our problem, Studies A, B, C and D are being continued. Study A is being reported in detail in the longer report.

Study A

Reattachment of detached soft tissue to the tooth and regeneration of surgically removed socket bone was reported in Study II of this series. However, the experimental con-

ditions differed from those encountered clinically. One of the main differences is that the periosteum from the bone removed remains on the detached gingiva. There is no periosteum on the detached gingiva in the conditions encountered clinically. To ascertain the role of this periosteum detached from the bone in the successful regeneration obtained in Study II is then of first importance, when considering the application of this work clinically. In Study A (this study) the role of detached periosteum is being studied by the use of an artificial substitute (polyethylene tubing). Again, autogenous grafts were also used in Study II and Study IV to aid osteogenesis. The use of autogenous grafts in dental practice is impractical. Bone grafts are used in orthopaedic surgery but largely on an empirical basis. Three types of bone grafts in common use are: autogenous compact bone, autogenous cancellous bone and heterogenous bone from bone banks. Differences in healing with the various types of grafts have been observed but the reasons for these differences are not yet clear. The use of a polyethylene tube permits a study of the role of grafts in osteogenesis, also of other factors concerned in bone repair. A better understanding of the role of grafts as aids in osteogenesis may facilitate the finding of a suitable substitute for use in dentistry. As these problems are also problems in surgery, Dr. I. MacNab, an orthopaedic surgeon is collaborating with us. This study is reported in detail in the long report.

Study B

In this study a direct effort is being made to secure reattachment and regeneration in epithelialized infected pockets without the use of grafts. It appears from examination of the living animal by means of a probe that some type of reattachment has been secured in about 35 days following the organization of a blood clot. However, confirmation of this finding must await histologic examination. Sections are being prepared; this study will be reported at a later date.

Study C

The purpose of this study is to ascertain the potentiality for cementum and bone repair of replacement cells originating from the connective tissues of the gingiva. Sections are being prepared in this study which therefore will be reported later.

Study D

As time permits, we are continuing the study of the effect of endocrines on the alveolar process of the rat, mentioned in the last report.

PROGRESS REPORTA Study of the Role of Detached Periosteum in the
Repair of Bone

The problem under investigation arose in the course of research on the regeneration of the supporting structure of the teeth lost through periodontal disease. In this investigation successful regeneration of supporting structures was induced where the gingiva had been detached and a section of socket bone and periodontal membrane had been surgically removed. These experimental conditions differ from those existing when the detachment and loss of structure is produced by periodontal disease. One of these differences is that in the surgically-produced lesions, the periosteum from the removed bone remains on the detached gingiva. There is no periosteum on the detached gingiva in the lesion resulting from periodontal disease. It became necessary therefore, to ascertain the role of the periosteum remaining on the detached gingiva when successful reattachment and regeneration was attained in the surgically-produced lesions.

The role of the periosteum in the regeneration of bone has been the subject of considerable controversy for over 200 years. It is recognized that the periosteum has a nutritive function insofar as it contains blood vessels concerned with the vascularization of bone. It is also believed to act as a limiting membrane preventing the escape of osteoblasts into the surrounding tissues. The dispute has concerned its bone forming properties. The problem is, therefore, to determine which if any of these important functions contribute to the success of the regenerative process when the periosteum remains after the bone has been completely removed. The possibility of an unknown function of the periosteum in osteogenesis must also be considered.

One of the experiments to investigate the osteogenic property of the periosteum, which was first used by Sir James Syme in 1837 and later by other workers, is of particular interest in the present study. Syme removed a section of the radius of both right and left legs in young dogs. On the right side he also removed the periosteum but on the left, this membrane remained in place. When the dog was autopsied it was found that the missing part of the left radius had been reproduced but on the right where the periosteum was removed a gap was left. From this and other experiments, he concluded that the periosteum was osteogenic. Fifty years later, Sir William McEwen repeated this experiment, but in his dogs the bone was not reproduced even when the periosteum remained. However, he was able to induce union by means of

grafts. McEwen believed that bone is a living tissue which reproduces itself, and that the grafts, rather than the detached periosteum, were the source from which the new bone grew.

The explanation now generally accepted (2, 3) to account for the conflicting results obtained by Syme and McEwen and other workers, is that in Syme's operations he must have dissected off both the outer and the inner osteogenic layer of the periosteum, while McEwen may have removed only the outer fibrous layer of periosteum. The difference of opinion is thus based on the precise definition of periosteum.

The results of preliminary studies in our investigation did not support this generally accepted explanation. McEwen's conclusions about the role of his grafts are also open to question. He used homogenous grafts, which are now believed to die and therefore could not act as the source of the new bone (4, 5). Since McEwen's time, grafts in various forms have been used to aid osteogenesis, but the manner in which they act is not yet clear. Grafts were used in our successful attempts to induce regeneration of the alveolar process in animals. But the use of grafts is not practical in dental practice. It was decided, therefore, to study carefully the roles of detached periosteum and of grafts in the regeneration of bone.

PROCEDURE

A gap of approximately 10 mm was made in the fibula of a dog by removing a section of bone and periosteum. Care was taken to ensure that all of the periosteum was removed with the bone. The fibula was selected since the gap is maintained by the intact tibia. The gap was bridged by polyethylene tubing, filled with autogenous cancellous grafts (small chips) from the iliac crest, and fitted over the cut ends of the bone. The wound was then sutured. Radiographs were taken at intervals. Union was completed in 118 days. As it was now evident from this pilot experiment that union would occur, a further study was carried out. A gap of approximately 20 mm was made in both fibula of twelve dogs, care being taken to remove all the periosteum with the bone. The experiments were divided into four groups. Group I - with one hind leg of this animal nothing was done except to suture the wound after removing the bone and periosteum. Using the other hind leg (Group II) polyethylene tubing was fitted over the cut ends of the bone bridging the gap and filled with blood. In a second dog, a polyethylene tubing was used on one leg as in Group II but the tubing was filled with autogenous cancellous grafts (small chips) from the iliac crest (Group III).

With the second hing leg of this dog the gap was filled with cancellous grafts as in Group III but tubing was not used (Group IV). Six pairs of dogs were completed in this manner:

Group I - following the removal of the section of bone and periosteum the wound was sutured.

Group II -- polyethylene tubing was fitted over the cut ends of the bone bridging the gap.

Group III- the polyethylene tubing was filled with autogenous cancellous grafts (small chips).

Group IV - the gap was filled with autogenous cancellous grafts. No tubing.

RESULTS

Five dogs became infected and were eliminated from the study, leaving 4 cases in Groups I, II, III and 3 cases in Group IV. Our original plan was to take radiographs every two weeks, but this interval was lengthened because four dogs died following the anaesthesia given for radiographic examination. However, histologic sections were made of these animals and the findings are included in the results. The remaining animals were sacrificed after 140 days. The number of animals was insufficient to permit definite conclusions regarding the results in the four groups and the findings recorded must be regarded as tentative and awaiting confirmation.

Group I - no grafts or tubing - 4 cases.

Histologic sections after 42 days were made of one dog. There was no regeneration of bone. Active resorption at one end of the shaft was still occurring. The gap was filled with fibrous tissue, the collagen-fibres arranged parallel to the long axis of the shaft. Some areas in the gap exhibited the characteristic appearance of tendon. Radiographs of the remaining three dogs autopsied at 140 days showed the gap still present or enlarged.

Group II - tubing only - 4 cases.

Due to technical difficulties with the tubing no histological regeneration of the bone with union in two of the three cases that survived to the 140th day.

Group III - tubing and grafts - 4 cases.

Three animals in Group III and IV were autopsied at 8, 28 and 63 days. Technical difficulties with the tubing prevented the preparation of histologic sections of the

animal at 8 days. The sections at 28 and 63 days showed considerable bone growth occurring in the gap. Radiographs of the fourth dog show complete union in 119 days.

Group IV - grafts only, no tubing - 3 cases.

Histologic sections at 8, 28 and 63 days showed bone growth occurring in the gap. The growth of bone was also quite apparent in the radiographs at 63 days. Union was not attained in this group.

Figures 1A, 1B, 1C, 1D and 1E are radiographs taken at 14, 28, 69, 90 and 119 days when the bone gap was bridged with polyethylene tubing filled with autogenous cancellous grafts (Group III). The gap in this case was about 10 mm. This was the animal used in the pilot experiment.

Figures 2a, 2b, 2c, 2d and 2e are radiographs of dog 12, representing Groups I and II, at 18, 27, 42, 56 and 140 days respectively. The right leg is Group I, and the left leg Group II. Note, on the left leg when tubing was used the regeneration of the bone with good union in the radiograph taken at 140 days. The gap still persisted on the right side (Group I).

Figures 3a and 3b are radiographs representative of Group III and IV at 9 and 49 days respectively. The left leg is Group III and the right leg is Group IV. Bone growth appears to have proceeded more rapidly when grafts were used (without tubing, Group IV) than when they were used with tubing. Histologic study of Group IV, presented later in this paper, suggests the reason for the more rapid growth. As yet union has not been obtained in this group.

Having found that bone could be grown down a tube with or without bone grafts we had available a method for investigating other fundamental problems in bone regeneration. The tubing presents some of the advantages of tissue culture, but used in a living animal it is more physiological. Blocks of tissue including both ends of the shaft and the contents of the tubing were removed from the animals that died, and were fixed in formol-saline. Corresponding blocks of tissue were removed where no tubing was used. Histological sections were cut longitudinally and stained with haematoxylin and eosin. Most of the animals that died belonged to Groups III and IV. The reports of the histological findings constitute the main part of this paper.

The usual method for investigating a biologic process by histologic means is to repeat the procedure in a number of animals which are sacrificed at various suitable time

intervals. We were fortunate in that when a tube was used the cellular invasion started from the cut ends of the bone and proceeded gradually down the tube. Therefore, the various stages in the regeneration of the bone could be examined in one section. For example, longitudinal sections of Dog 13, Group III, after 28 days showed bone formed at both ends of the tubing, while in the centre of the gap, grafts were still lying in the cot. The various steps in the formation of bone between these stages could thus be observed in one section.

A series of photomicrographs (Fig. 4 to 22) from Group III illustrates the stages of bone growth in the tubing starting at the centre of the gap and proceeding towards one end. Most of the fields shown here are from one section at 28 days. A few photomicrographs from a section at 63 days are included where a certain stage in the regenerative process is particularly well illustrated. Figure 4 is a view of the tissue in the tubing about midway between the cut ends of the bone, showing a graft still lying in blood clot. In Figure 5, a little nearer to the end of the tubing, we see that blood vessels and cells are invading the clot. In Fig. 6 a little farther along the tubing the clot has been resorbed and replaced by a relatively undifferentiated mesenchymal tissue. Fig. 7 shows the grafts lying in this undifferentiated tissue which appears to have become attached to the grafts. This can be more clearly observed in Figure 8, a higher magnification of the rectangle in Figure 7. The striated appearance of the border of the grafts is seen where the direction of the collagenic fibrils of the bone is disposed roughly at right-angles to its surface. Notice that the collagenic fibrils in the graft are continuous with those in the striated border. Note also that the cells and intercellular fibrils of the new tissue have grown into the striated border. The whole picture is consistent with the hypothesis suggested by Ham (6) that striated borders are caused by the mineral contents of the bone dissolving before the collagenic fibrils do, leaving the fibrils free to project a short distance from the surface of the bone. They in turn appear to melt away at their free ends at about the same rate as the mineralized element dissolves so that the striated border never becomes very thick.

As we go down the tubing (still Group III), the next stage is shown in Figure 9, the appearance on the scene of osteoclasts. Notice the moth-eaten appearance of the grafts caused by Howship's lacunae in contrast with the smooth appearance of the bone in Figure 7 before the osteoclasts appeared. In Figure 10, osteoclasts are

appearing in the tissue some distance from the bone. However, the possibility of a graft being present a few microns deeper into the block must be kept in mind. Figure 11 is a high magnification of an osteoclast showing a ciliated border. Figure 12 shows an osteoclast stained with Mallory's connective tissue stain which stains the osteoclast red and the bone blue. Even in the black and white micrograph the cilia appear to be protoplasmic extensions of the osteoclast growing into the striated border of the bone. Osteoclasts are short-lived. Figure 13 shows a degenerating osteoclast with characteristic vacuolization. Figure 14 shows the initial formation of new bone growing as coarse trabeculae on the previously resorbed surfaces of the grafts. The osteoclasts have disappeared and the picture is one of apposition rather than resorption. Figure 15 is a higher magnification where the contrast between the empty trabeculae of the graft and the deep-staining osteocytes in the new bone is quite distinct. Figure 16 shows a graft on which a considerable amount of new bone has been laid down and now for some unknown reason the cells instead of differentiating into osteoblasts are becoming chondroblasts and forming cartilage. Figure 17 is a later stage where a cartilage bridge has been thrown across the space between the new bone on one graft to the new bone on another. New bone seems to be formed on the grafts first, before the cartilage. The conditions are evidently unsuitable for the maintenance of cartilage as it soon breaks down (Figure 18). In Figure 19, a large osteoclast is seen lying in contact with a remnant of the cartilage. The resorption of the cartilage is followed by another wave of osteogenesis. In Figure 20, bone is being laid down on the remnants of the cartilage, similar to the bone growth on the diaphyseal side of the epiphyseal plate in a growing long bone. The cartilage is completely replaced by bone in Figure 21. But in Figure 22 we see that the process of resorption and apposition is still going on. The new bone is now being resorbed and more new bone is being formed.

Figures 23 and 24 are of sections of Group IV where grafts alone were used to fill the gap. Figure 23, a low magnification at 8 days shows a graft about midway in the gap. The higher magnification in Figure 24, shows that a considerable amount of new bone has been formed around the graft.

SUMMARY OF FINDINGS

1. Where a gap was made in the fibula of a dog by removing a section of bone with its periosteum, the bone was not reproduced (4 cases).

2. When the gap was filled with autogenous cancellous grafts, (small chips) considerable bone regeneration occurred (3 cases).
3. Again when the gap was bridged with polyethylene tubing, there was complete regeneration of the bone, the presence or absence of grafts in the tubing apparently made little difference in the end result or the rate of regeneration.
4. Bone was reproduced faster where autogenous grafts (cancellous) were used without tubing than with tubing alone or tubing filled with autogenous grafts but union was not obtained. Further work is required to ascertain whether bone union can be induced by means of autogenous cancellous grafts alone.
5. The manner in which bone was formed in the tubing filled with grafts appeared to be different in some respects from that followed when cancellous grafts were used without tubing.
6. The histological observations suggest two types of resorption of autogenous grafts.

DISCUSSION

The role of periosteum detached from the bone was studied by providing an inert substitute, polyethylene tubing. While the nutritive and osteogenic functions of detached periosteum may have some part in osteogenesis, the fact that the bone was reproduced when the tubing was used suggests that it may have other functions. It may play the role of a limiting membrane, or exert some unrecognized effect. The periosteum as a limiting membrane may function by guiding the osteogenic cells or by preventing other cells from proliferating into the area.

Comparison of the rates of bone reproduction in the radiographs 3a and 3b, representing Groups III and IV, indicates that regeneration was faster where grafts were used without being enclosed in tubing. The histological findings more or less explain this observation. The amount of new bone in Figure 24, Group IV, formed from the graft in 8 days is surprising. The appearance and amount of the new bone suggest that some of the covering and lining cells of the graft survived and proliferated directly or almost directly into fully differentiated osteoblasts. Some of these began to lay down bone immediately while others continued to proliferate and form more osteoblasts. Under these conditions, cells specialized to form bone are seeded all along the gap and result in

rapid growth of bone. Thus one way is apparent by which autogenous cancellous grafts may aid osteogenesis. However, homogenous grafts from a bone bank are known to aid in bone reproduction (7, 8). It could hardly be expected that such cells would survive and proliferate (4, 5). There must be some other mechanism by which these grafts aid osteogenesis ^{*}.

The cycles of resorption followed by apposition described in the growth of bone within the tubing (Figures 7 to 22) present an interesting sequence of events. We saw that, following the organization of a blood clot there is resorption of the grafts followed by a deposition of bone on the resorbed surface. This appositional growth peters out, to be followed by chondrogenesis. The cartilage dies and is resorbed. Apposition on the remnants of the resorbed cartilage then occurs. Resorption of the new bone occurs again followed by apposition. It almost appears as if appositional growth of bone is self-limiting. Some stimulus resulting from resorption of calcified tissue, bone or cartilage, seems to be required before another wave of appositional growth is initiated. The potential usefulness of such a mechanism is apparent.

A few years ago the author observed this cyclic phenomenon in a series of cases in another study. Following the removal of a section of bone from the maxilla, new trabeculae of bone growing from the cut surface were seen in the histologic sections. This growth of bone soon stopped and the rest of the space had filled with undifferentiated cells. There was no further bone growth. Under the same conditions, when grafts (small chips) were used to fill the space, the result was bone reproduction instead of soft tissue replacement. We suggested that the resorption of the grafts seemed to supply the stimulus for the continued growth of bone.

It is of interest to note that in Group IV where grafts were used without tubing, after the first wave of osteogenesis (Figure 24) cartilage was formed in a manner similar to that occurring in the tubing. From that stage on, the growth of the bone followed the same pattern as in the tubing. Our explanation for the additional cycle observed within the tubing (Figures 4 to 7) is that none of the cells

^{*} From the findings on these and previous studies, the writer is attempting to build up a concept of the manner in which bone defects are repaired and the factors essential in the reparative process. The ideas advanced in this paper form a working hypothesis on which to base further work and are not considered to be conclusions. Experiments are being planned to test the various aspects of the theory set forth.

of the grafts survived, as the tubing effectively prevents any nutrition from the blood vessels in the area. Thus after the first wave of osteogenesis at the ends of the bone, the cells continued to proliferate around the grafts as undifferentiated cells. Not until resorption of the grafts initiated what actually was a second wave osteogenesis, did appositional growth occur farther down the tubing.

It thus appears that in the repair of a defect in bone after the first wave of osteogenesis, if cellular proliferation continues, the cells either differentiate to chondroblasts or continue to proliferate as undifferentiated cells. If differentiation to chondroblasts takes place, the end result will be the formation of bone following the resorption of the cartilage. If, however, the cells continue to proliferate as undifferentiated cells and there is no calcified tissue (grafts) to be resorbed to initiate another cycle of appositional growth, the probable result will be fibrous tissue replacement.

The mechanism of bone growth described suggests the role in osteogenesis of the detached periosteum and of the tubing. Their role may be the maintenance of space by preventing either the collapse of the soft tissues into the wound or the active invasion of cells from the neighbouring soft tissues. In either case the processes necessary for the formation of bone across the gap would be interrupted.

What, then, is the role of grafts? They may aid osteogenesis in three ways: 1) by providing dead bone for resorption and 2) by maintaining the space as does the tubing. 3) Autogenous cancellous grafts (small chips) can have the additional advantage of seeding osteoblasts along the wound. On this basis it is evident that the various types of grafts have advantages and disadvantages. For example, well fitted solid compact grafts would be more effective than cancellous grafts (small chips) in (2) maintaining the space. However, compact grafts have the disadvantage of greatly slowing up the infiltration of osteogenic cells and thus replacement of the grafts with living bone would be slow. Used as solid compact grafts, autogenous grafts would have little advantage over homogenous bone as few of the covering or lining cells of the autogenous compact grafts would survive. Autogenous cancellous grafts would be superior to homogenous (4, 5, 9, 10) in the type of defect where a significant number of covering and lining cells could be expected to survive. Their rapid proliferation would also tend to prevent invasion of the cells from the soft tissue. But when conditions are such that few grafts cells survive, autogenous grafts would have little advantage.

over the homogenous type. The surgeon then would determine the most suitable grafts according to the type of defect.

If resorption is essential in the growth of bone, the mechanism by which bone is resorbed takes on additional interest. Since the time of Kolloker, it has generally been believed that bone is built by specialized cells, osteoblasts, and destroyed by specialized cells, osteoclasts. The appearance of the striated borders of the grafts in the tubing before osteoclasts had formed, suggests that resorption of the grafts may occur by mechanisms other than by the action of the osteoclasts. This observation supports the view expressed by Ham (11) that resorption of bone may be the result of failure of maintenance of bone. However, the moth-eaten appearance of the grafts due to Howslip's lacunae where osteoclasts are present (Fig. 9) in contrast with their smooth outline where undifferentiated cells are contacting the grafts (Fig. 7) favours the classical view of osteoclastic resorption.

Mallory (12) observed that the cytoplasmic borders which osteoclasts present to bone often appear to be striated. This phenomenon is well illustrated in Figure 11. Ham (9), however, believes that the "so-called striated borders of osteoclasts are nothing more than the fringed surfaces of bone at sites where bone is dissolving away." Our findings, illustrated in Figure 8, agree with Ham's view on the cause of striated borders on bone, but we believe that when osteoclasts come into contact with such a border cytoplasmic processes grow between the collagenic fibrils (Figure 12) as did the undifferentiated cells in Figure 8.

Having attained successful bone union across the gap by the use of the polyethylene tube, the question arises: What is the role of the tube in the reparative process? In Group I (no grafts or tubing), it was apparent that the muscle had collapsed in the gap. In which case, the main function of the tube may be the preservation of space. To test this hypothesis, the following study was carried out.

PROCEDURE

The same procedure described in the preceding study, of removing a section of bone with its periosteum from the fibula, forming a gap of about 20 mm, was used. Polyethylene tubing was used to bridge the gap as in the previous study, with one difference. Holes were cut in the tubing - care being taken to leave sufficient strength so that it would still prevent the collapse of the muscles

into the gap, thus preserving the space. This procedure was carried out on one leg of five dogs. The other leg was used for a purpose that will be referred to later. In addition, perforated tube was placed in the muscle of the back. Radiographs were taken at intervals throughout the study. The animals were sacrificed after approximately 100 days and blocks were removed for histological section.

RESULT

Both radiographs and histological sections indicated that the replacement was of fibrous tissue in all cases. The photograph (Figure 25) of the tissue replacement in the gap was taken after the perforated tube was removed. The space from which the tubing was removed and the effect of the perforation is clearly visible.

Figure 26 is a low-power view of the tissue in a perforation. Notice that the muscle is present through the perforation and in the connective tissue in the tube. The question is, did the muscle grow through the perforation into the tubing or did it bulge into the perforation following the operation. This is an interesting observation if it grew in, as it is unusual for skeletal muscle to grow in this manner. However, that is outside of the problems we are concerned with in this study. Figure 27 is a medium-power magnification, while Figure 28 is a high-power magnification in the area in Figure 26.

DISCUSSION

It appears that bone is not formed in the tube when perforated. The perforated tube prevented collapse of the tissue into the gap, thus maintaining the space as well as the intact tube. It seems then that the tube must function in some other manner. It can be inferred that the tubing either prevents something undesirable from coming into the tube, or prevents something desirable from leaving the tube. For example, the perforations might permit fibroblasts from the lateral walls to grow into the tube. Either through lack of osteogenic potency or suitable environmental stimuli, differentiation of cells from this origin does not take place. Again, another explanation of the findings in this study may be that a suitable environment for osteogenesis is established and maintained by the tubing. Perforation of the tubing, permitting a free interchange of fluid between the inside and outside of the tube, prevents the establishment and maintenance of a correct environment for osteogenesis. Experiments are being planned to attempt to ascertain if either of these logical interpretations are correct.

Another study is being carried out arising from the findings ascertained from certain procedures carried out on the remaining leg of the five dogs. Briefly, the purpose was to clarify the requirements for successful bone growth using the tube. Four dogs are used in this study. On one leg, a slightly larger intact tube with grafts is being used to bridge the gap. On the other leg the same intact tubing is used but without grafts. This will also permit a comparison of the rate of repair under these different conditions.

In the study on the remaining leg of the five dogs, the tubing used fitted the fibula more closely than was used in the previous studies. The result was that there was little bone regeneration under these conditions. Possibly the periosteum was unable to become elevated by the cellular proliferation when the closer fitting tube was used, thus interfering with the source of the cells. Dr. McNab carried out a study with his group that seems to support this interpretation. The role of the blood clot itself in bone repair is also being studied in this experiment.

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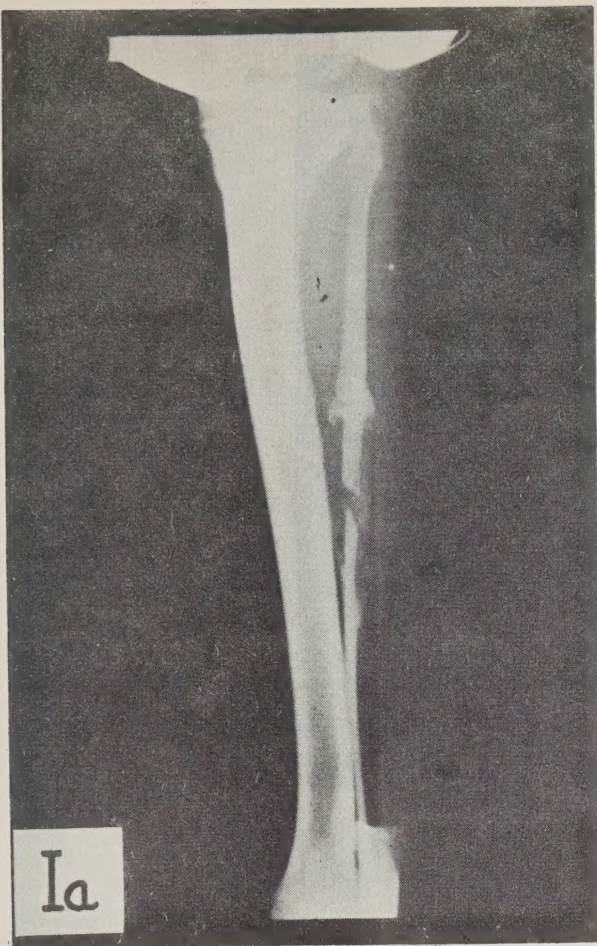
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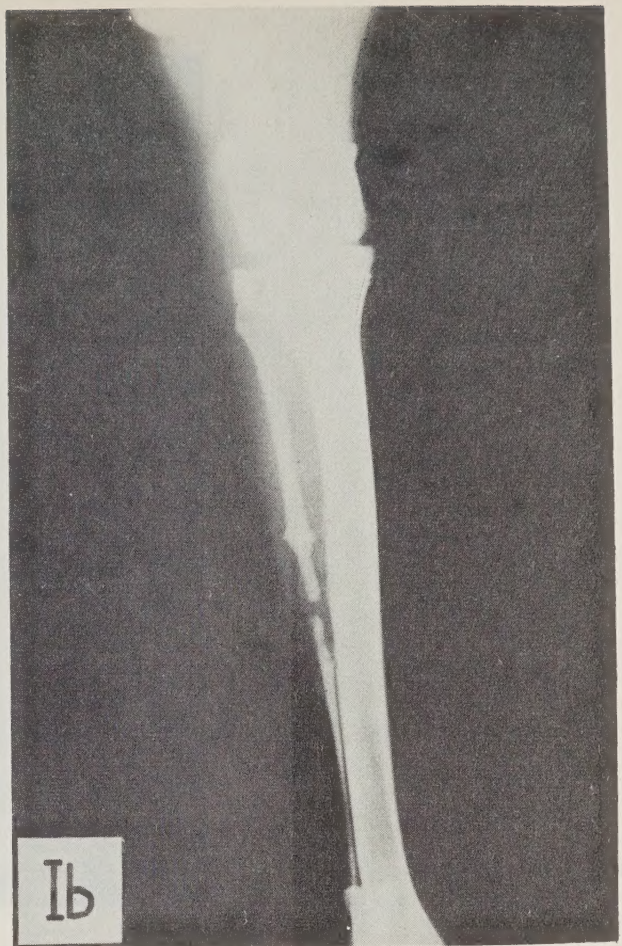
At 14 days

At 21 days

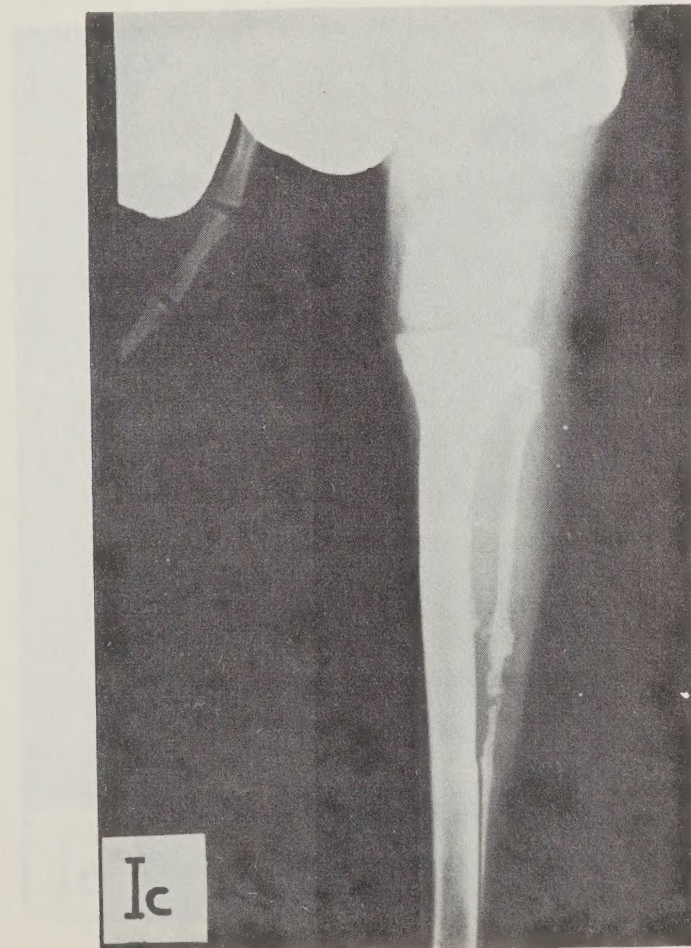




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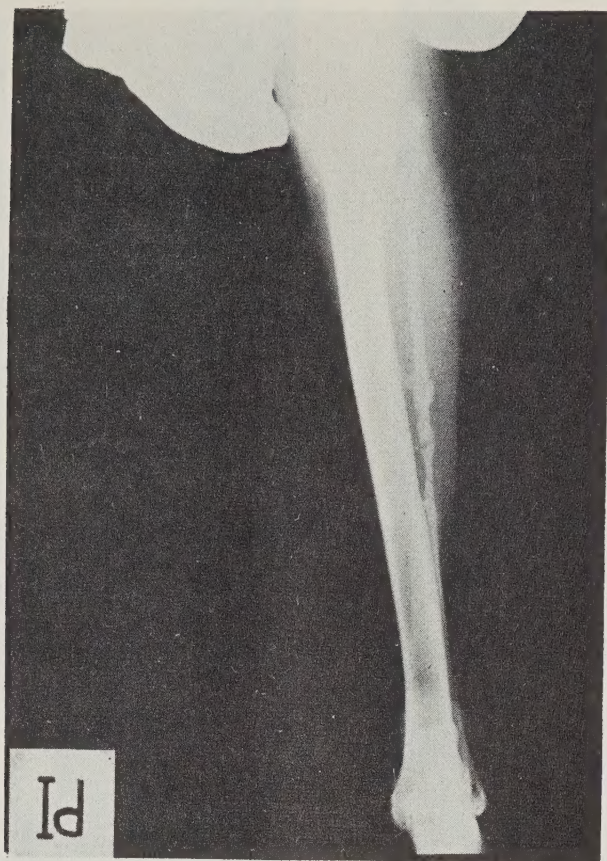


At 28 days

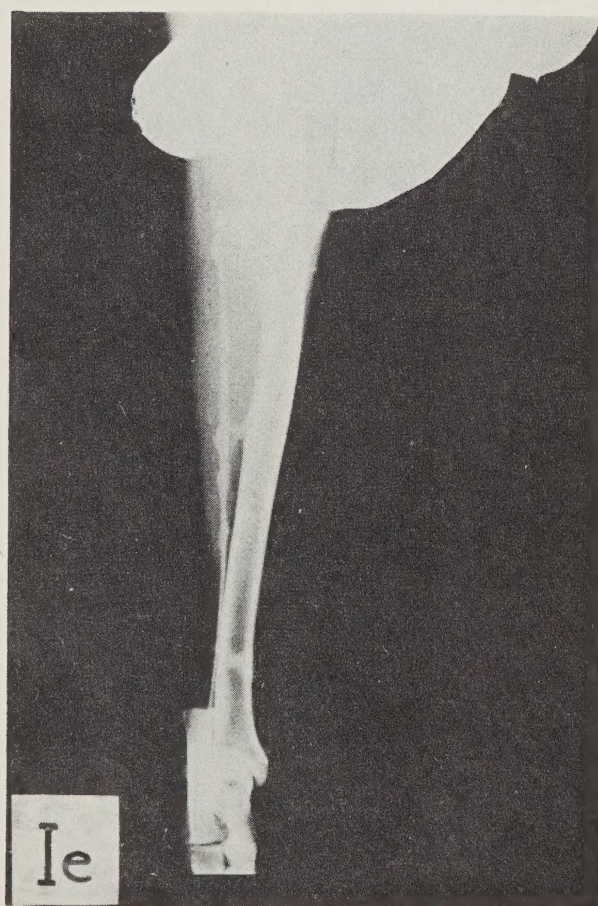


At 69 days

Group 3

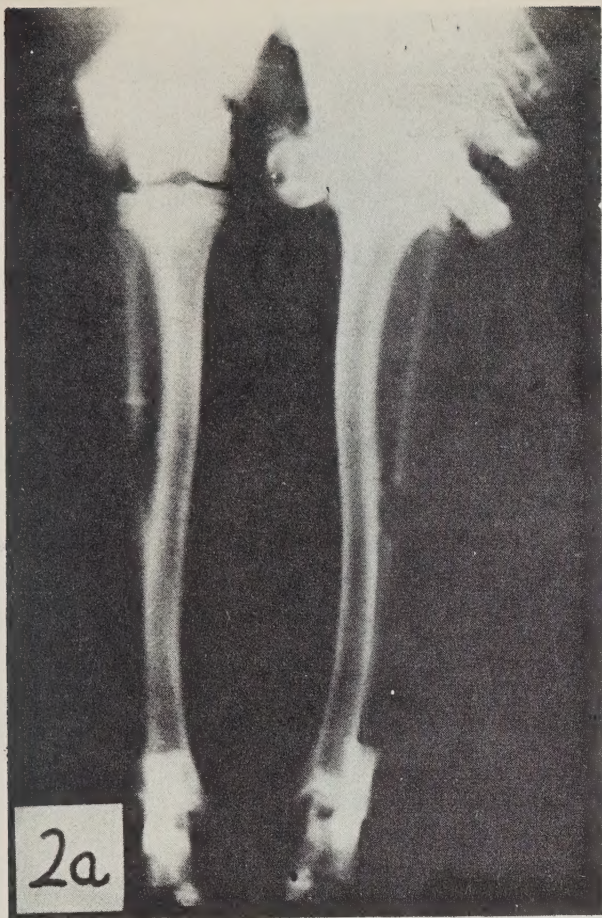


At 90 days

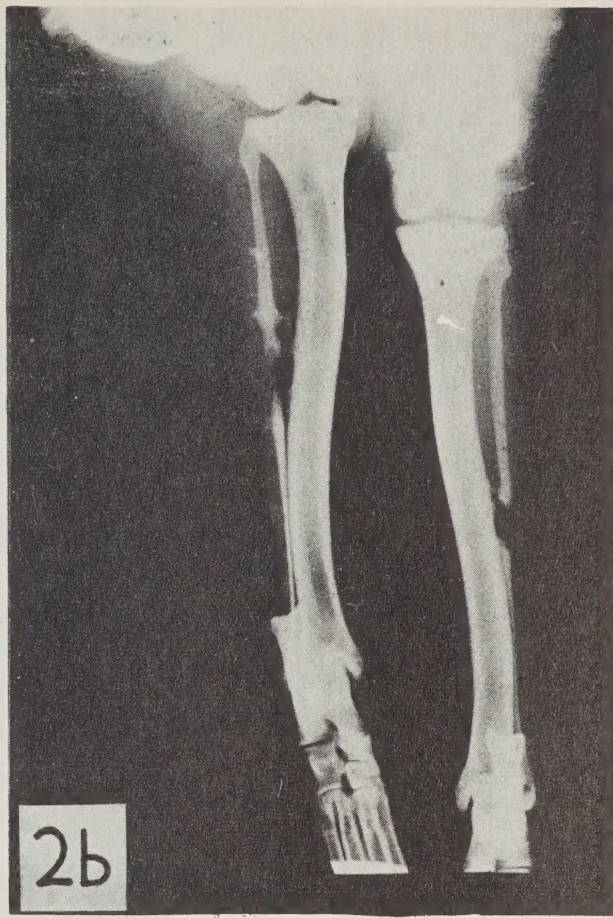


At 119 days

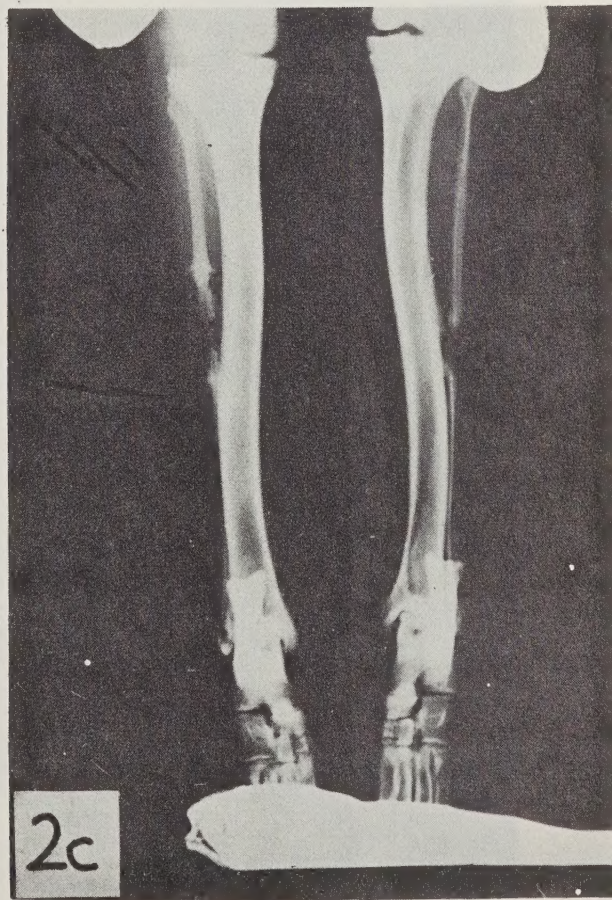
Group 3



At 18 days



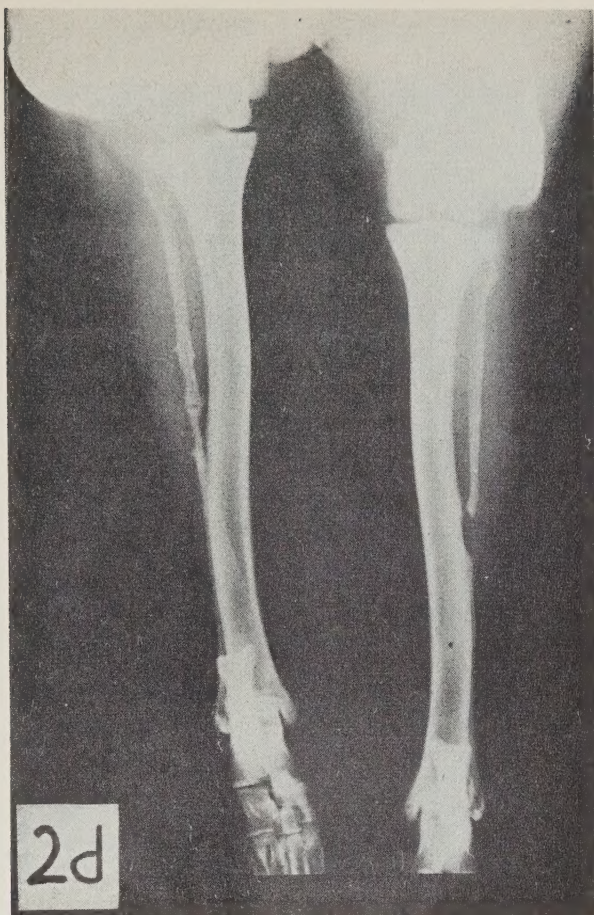
At 27 days



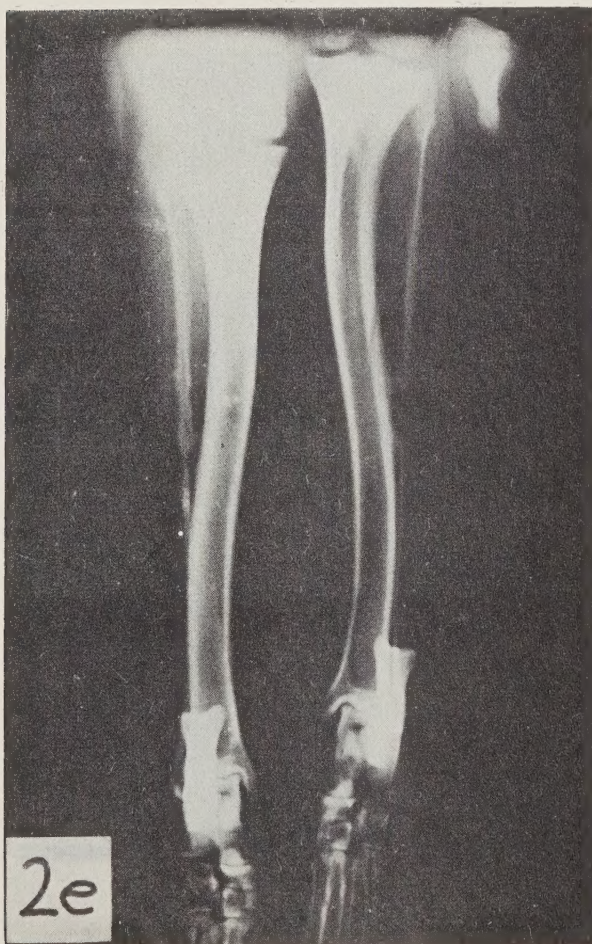
At 42 days

Group 1 - Right Leg

Group 2 - Left Leg



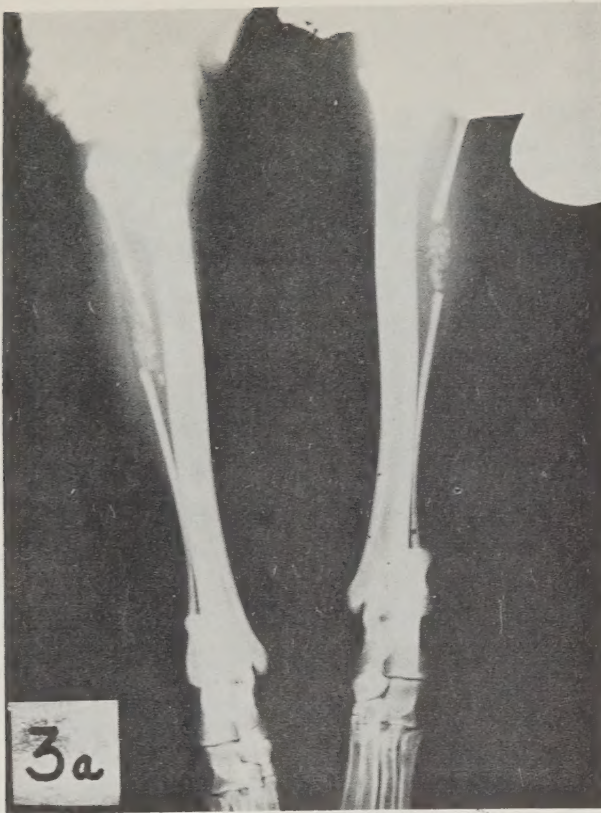
At 56 days



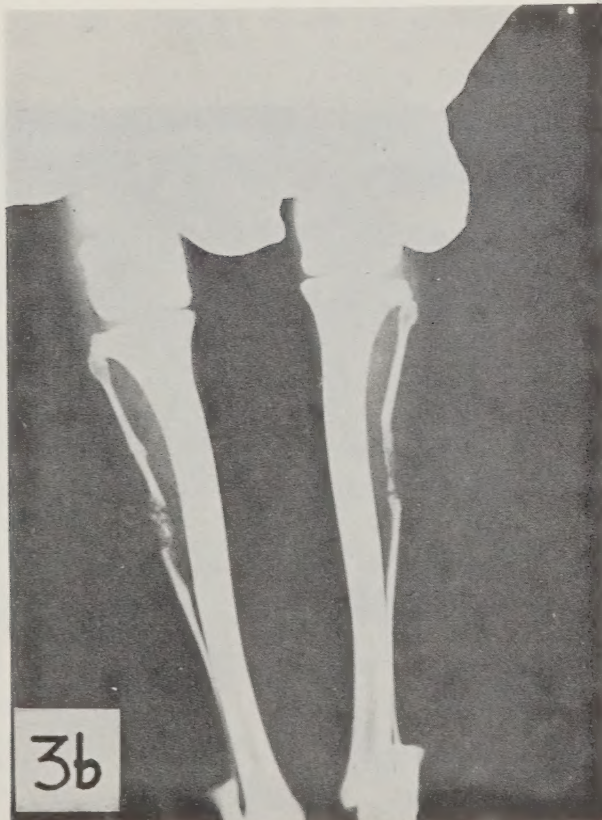
At 140 days

Group 1 - Right Leg

Group 2 - Left Leg



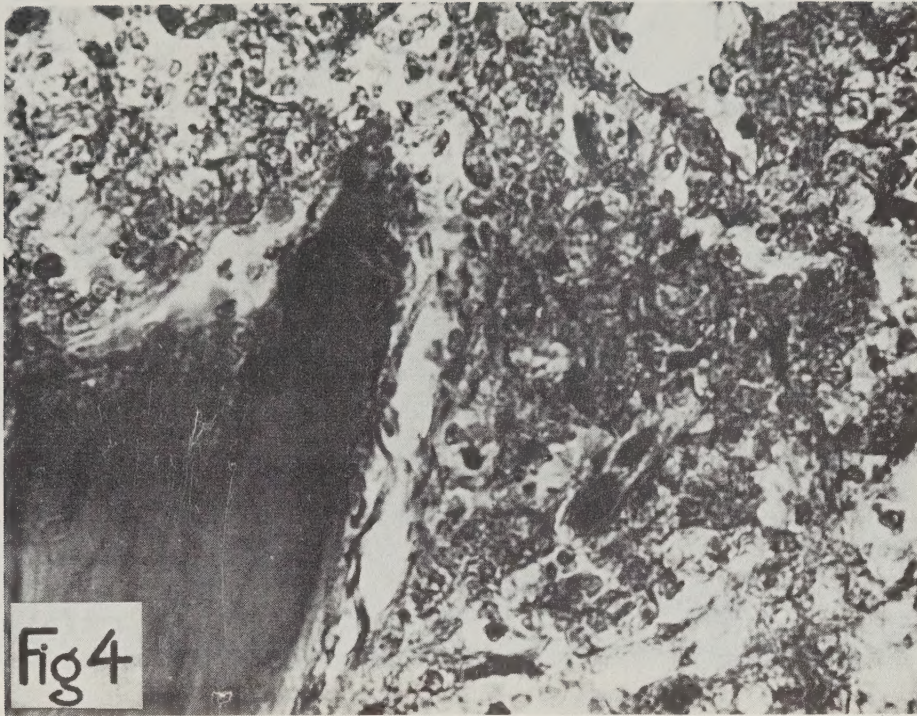
At 9 days

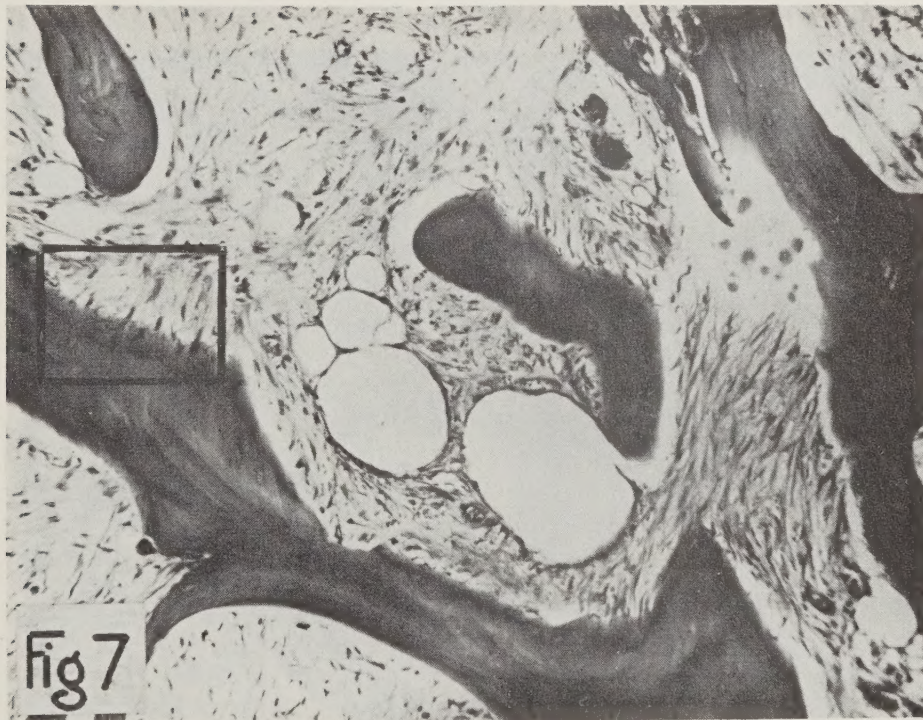
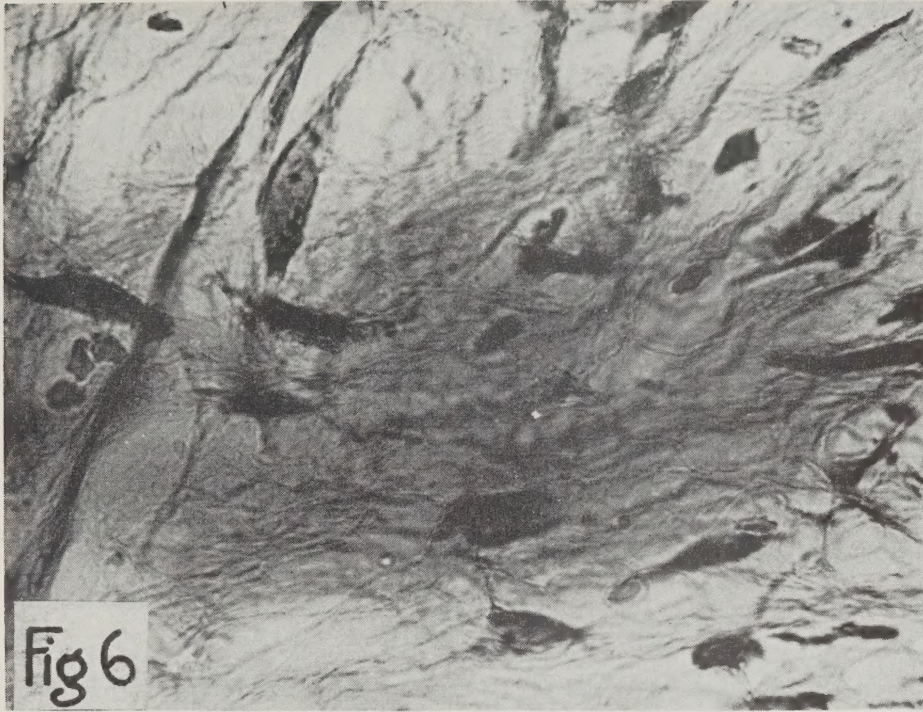


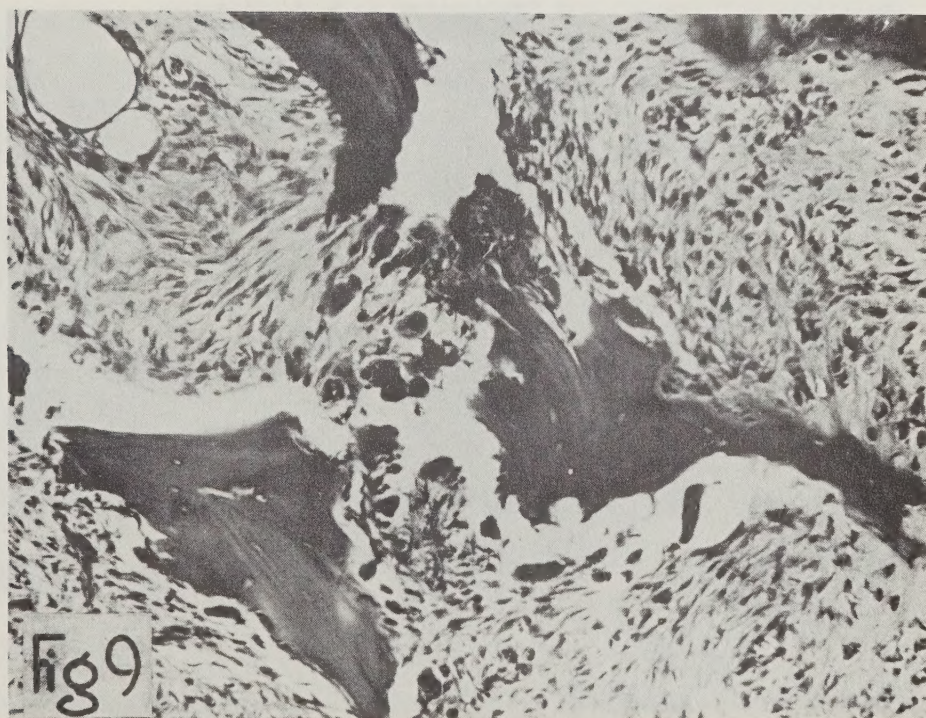
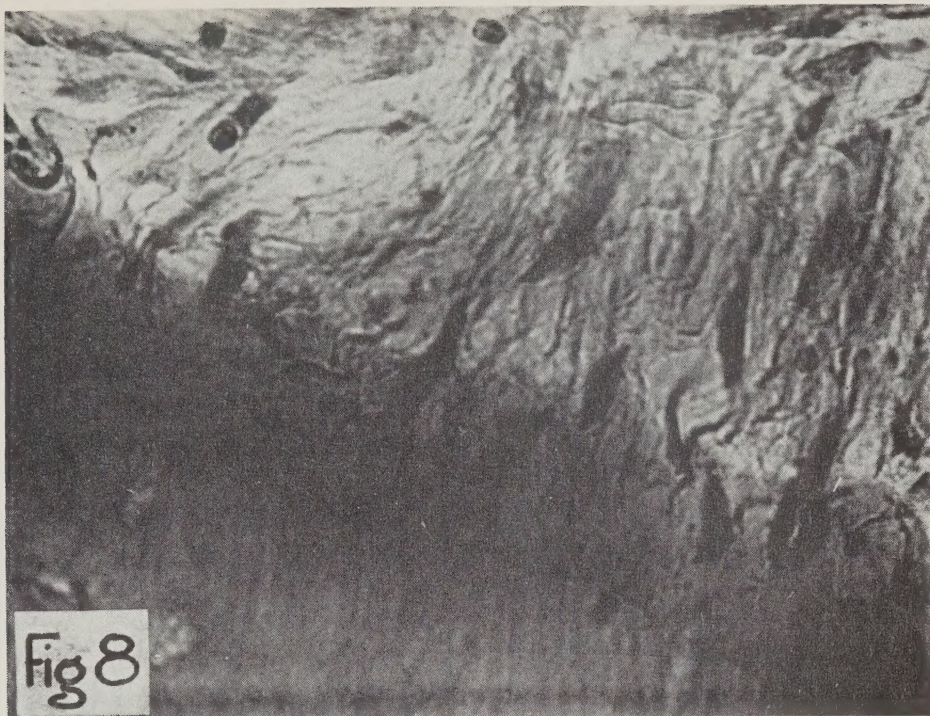
At 49 days

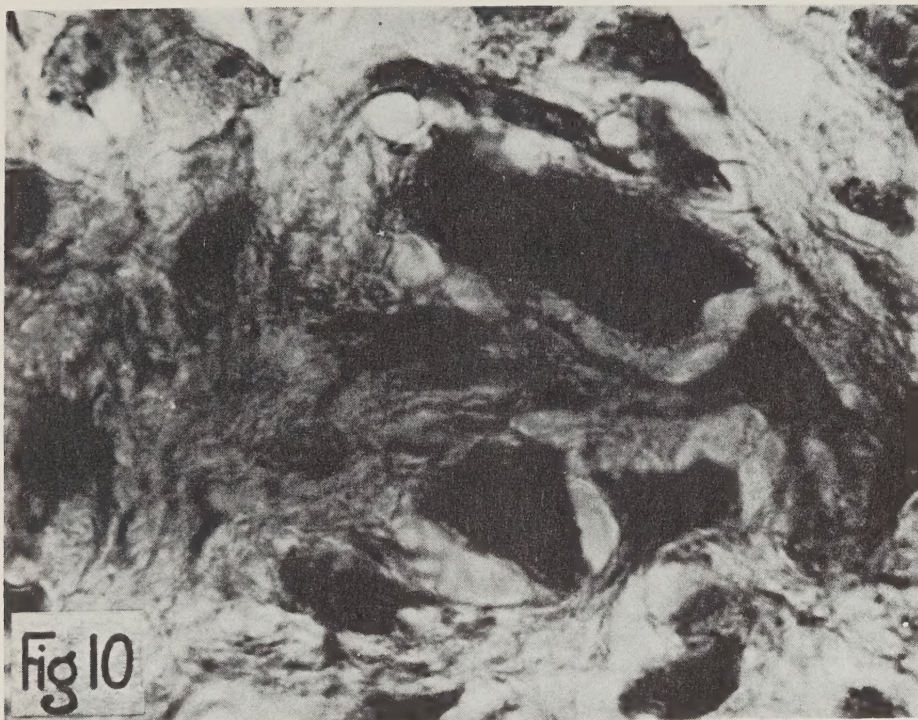
Group 3 - Left Leg

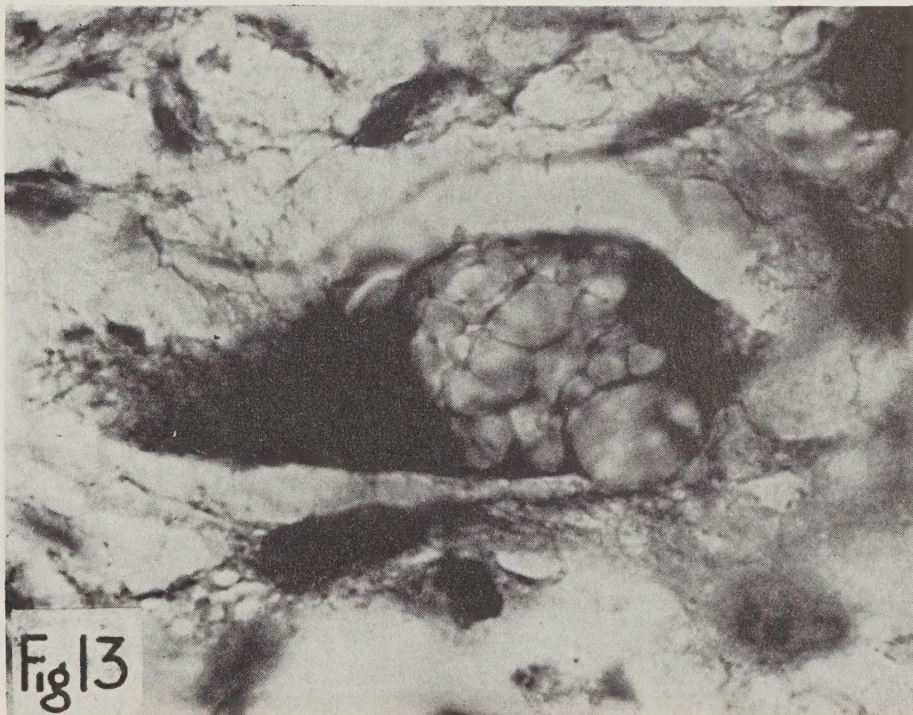
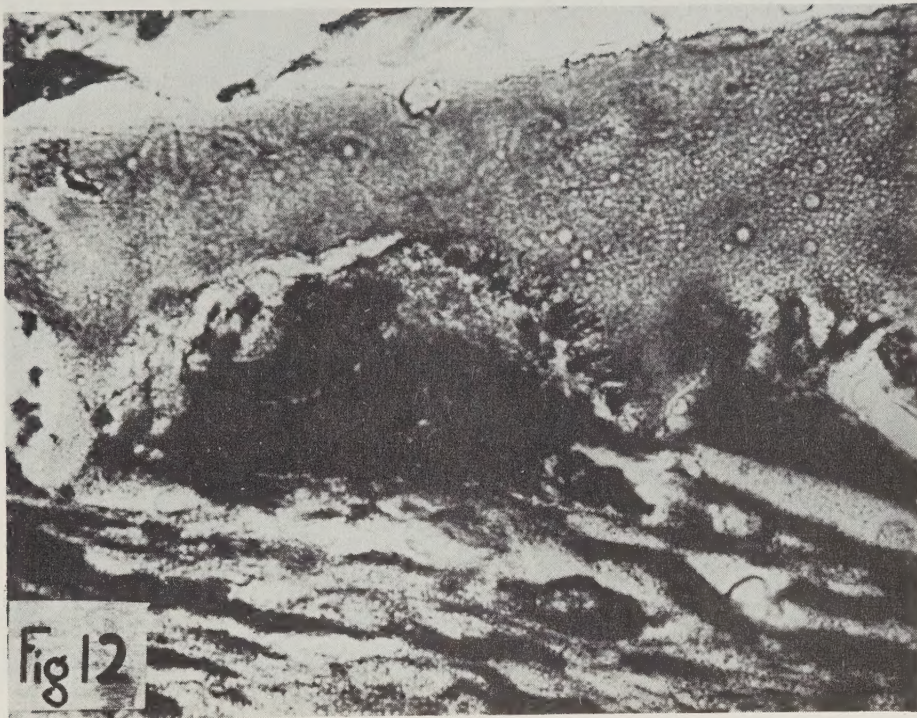
Group 4 - Right Leg

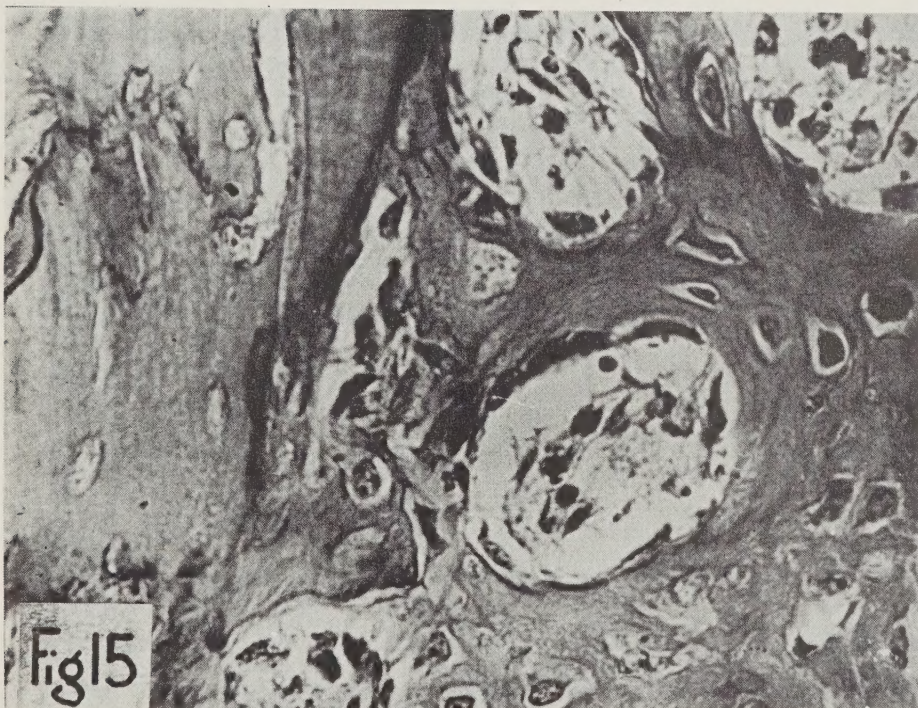


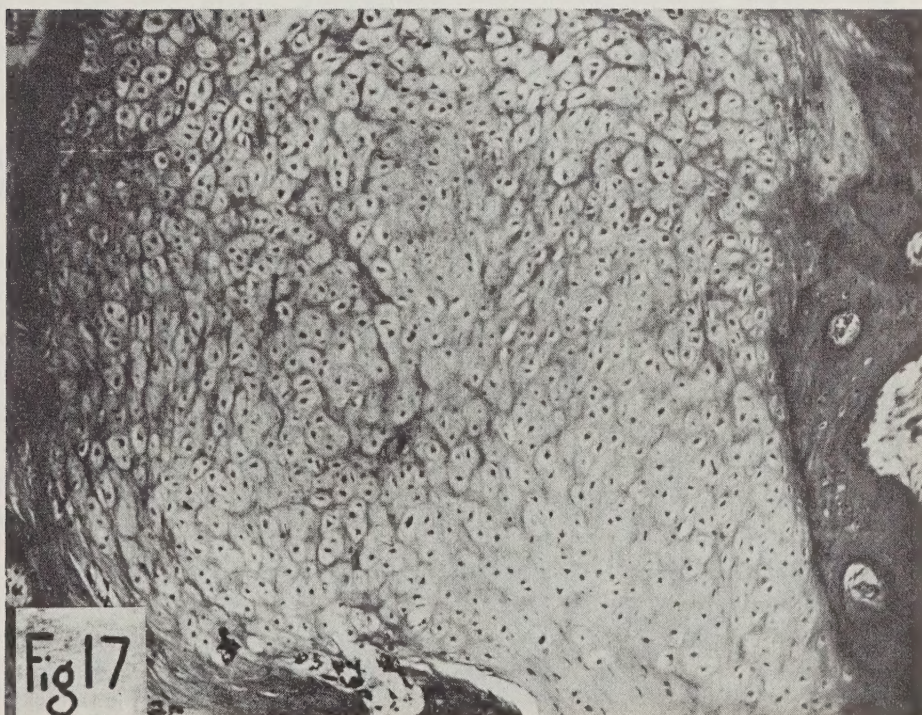
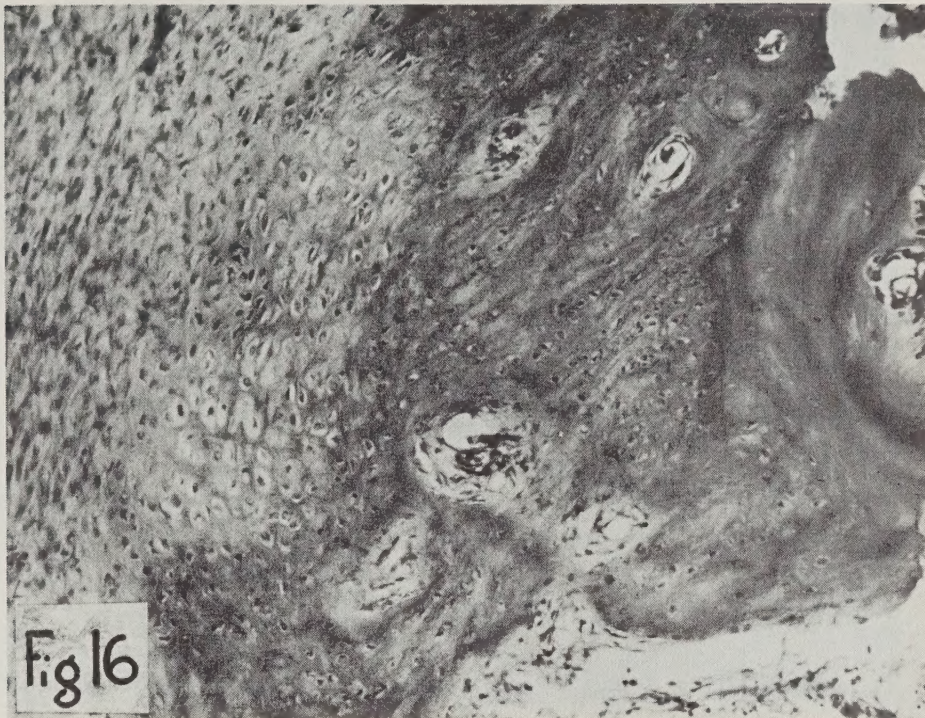


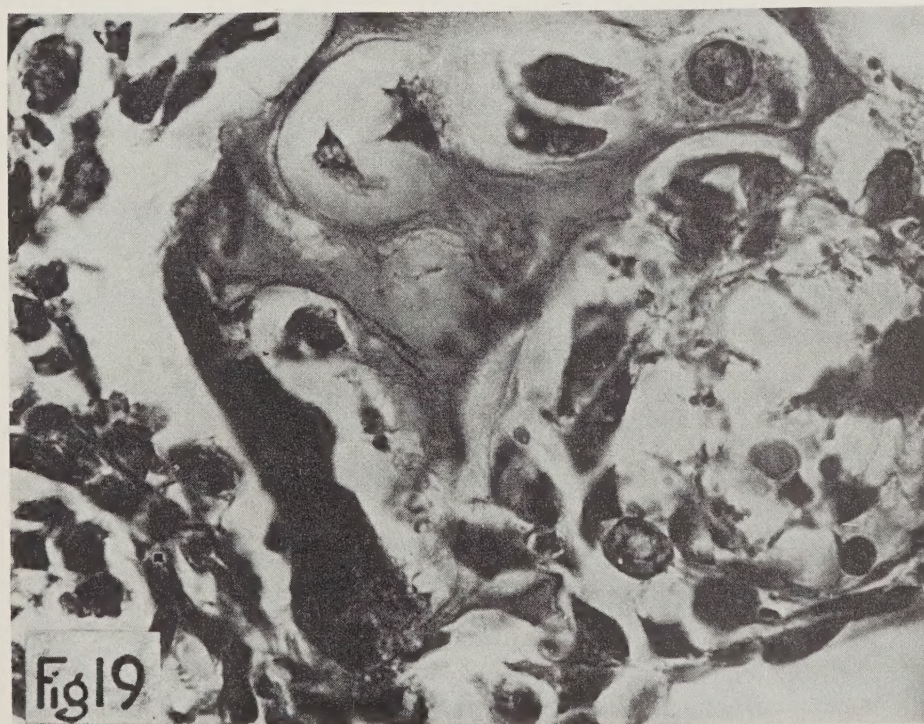
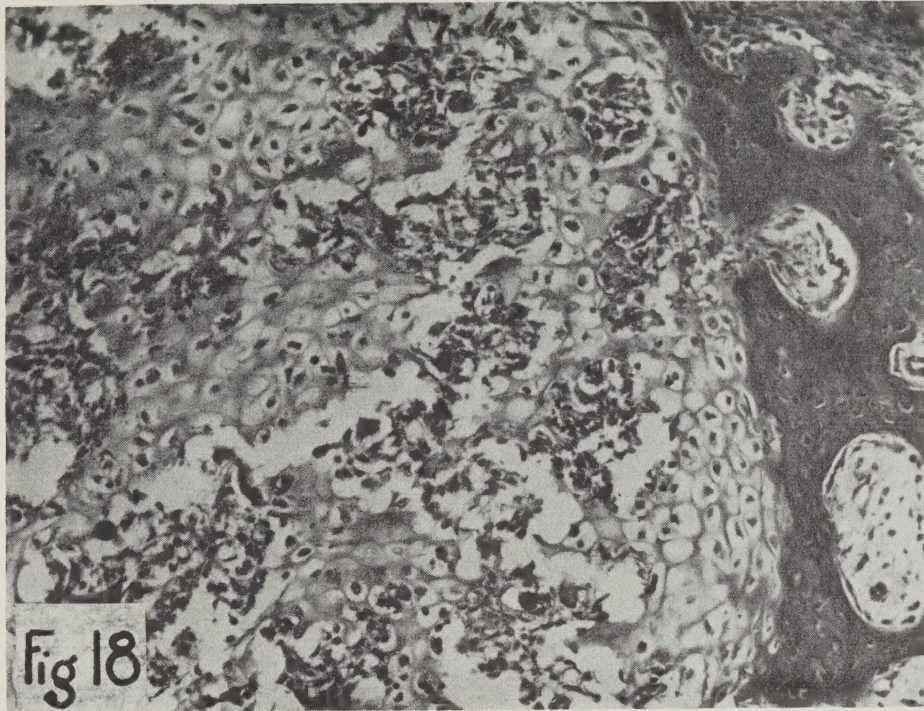


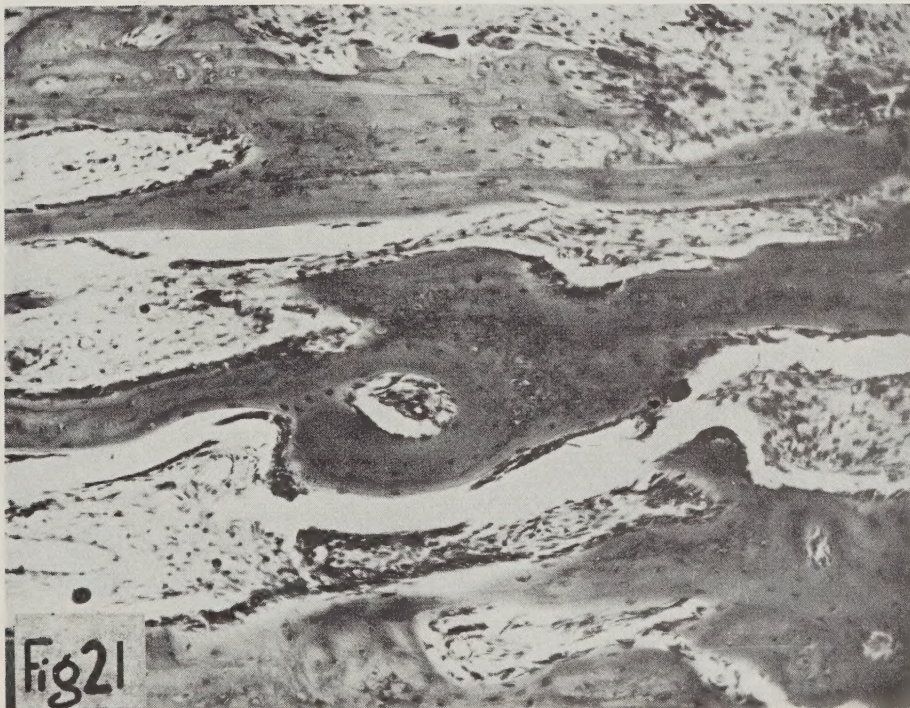
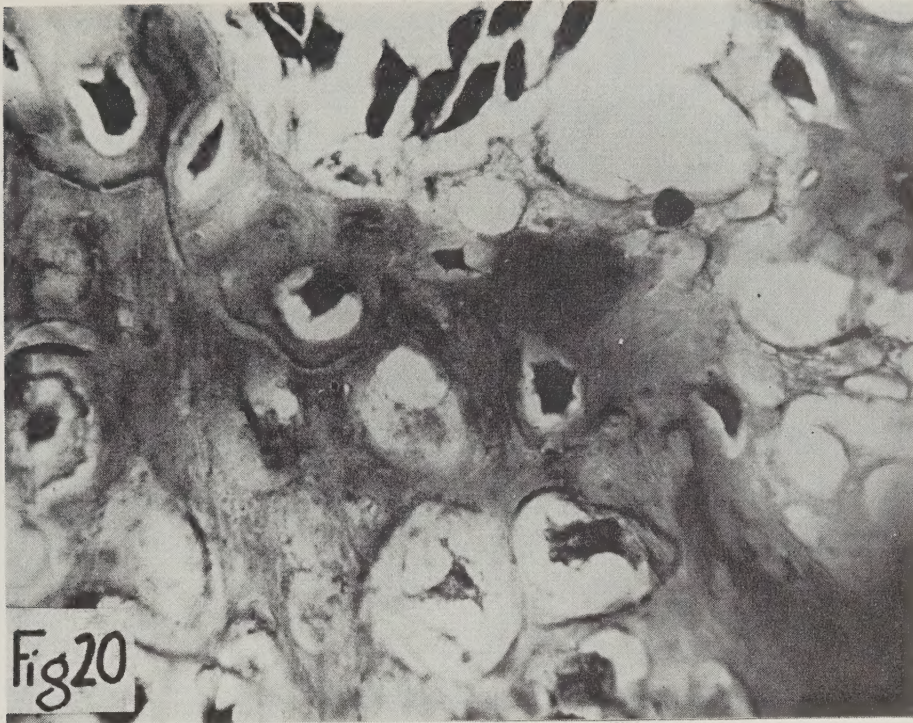












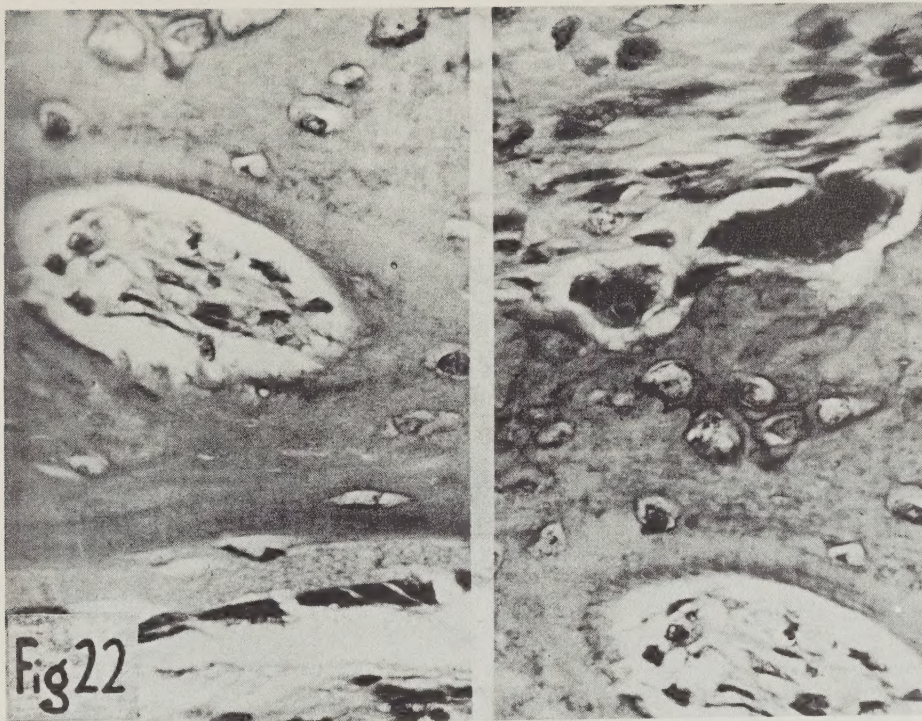


Fig 22

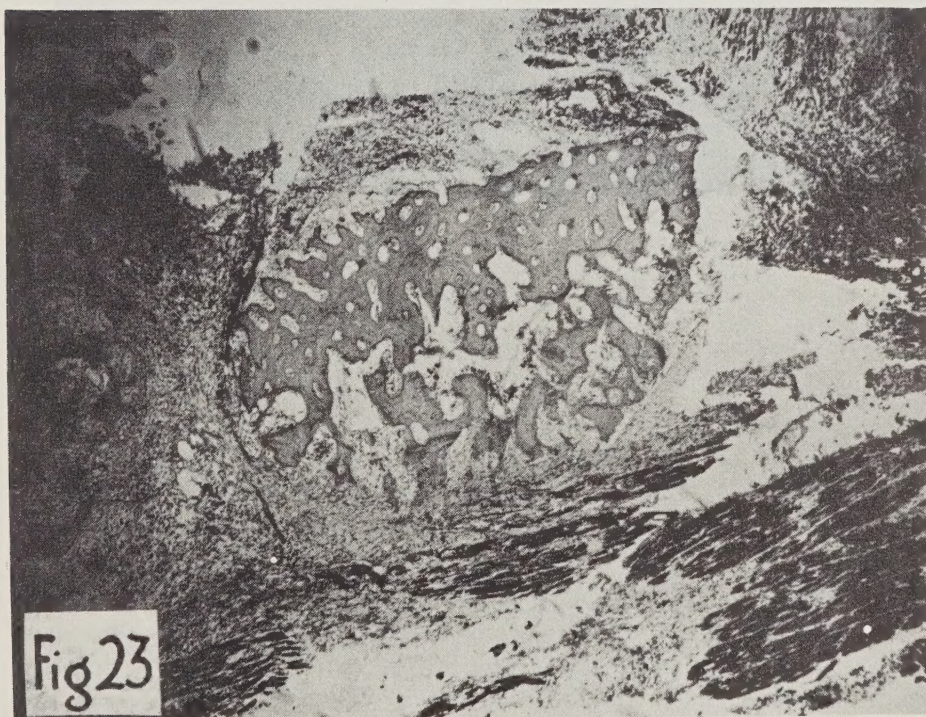
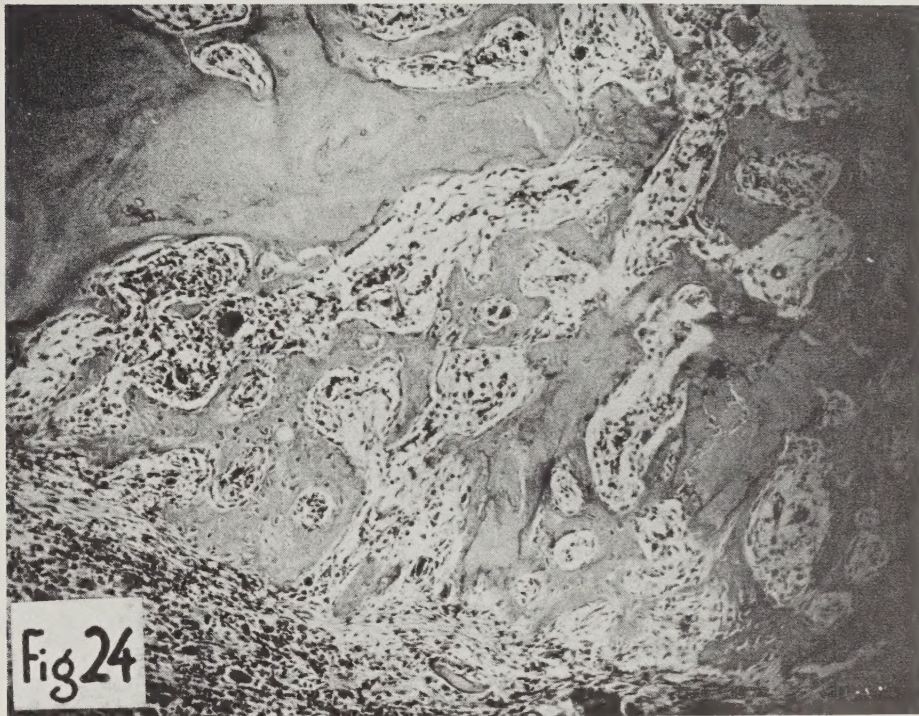
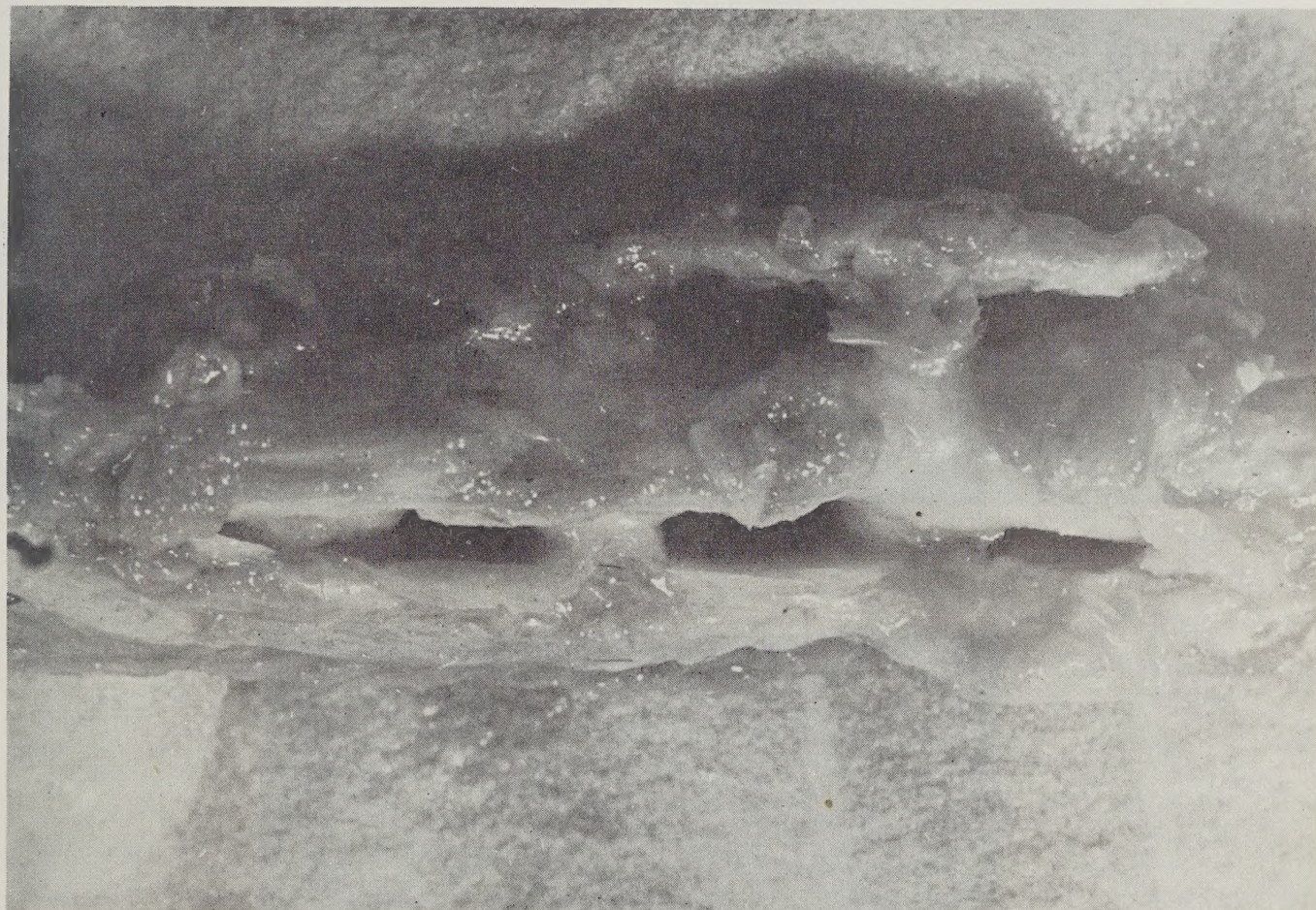


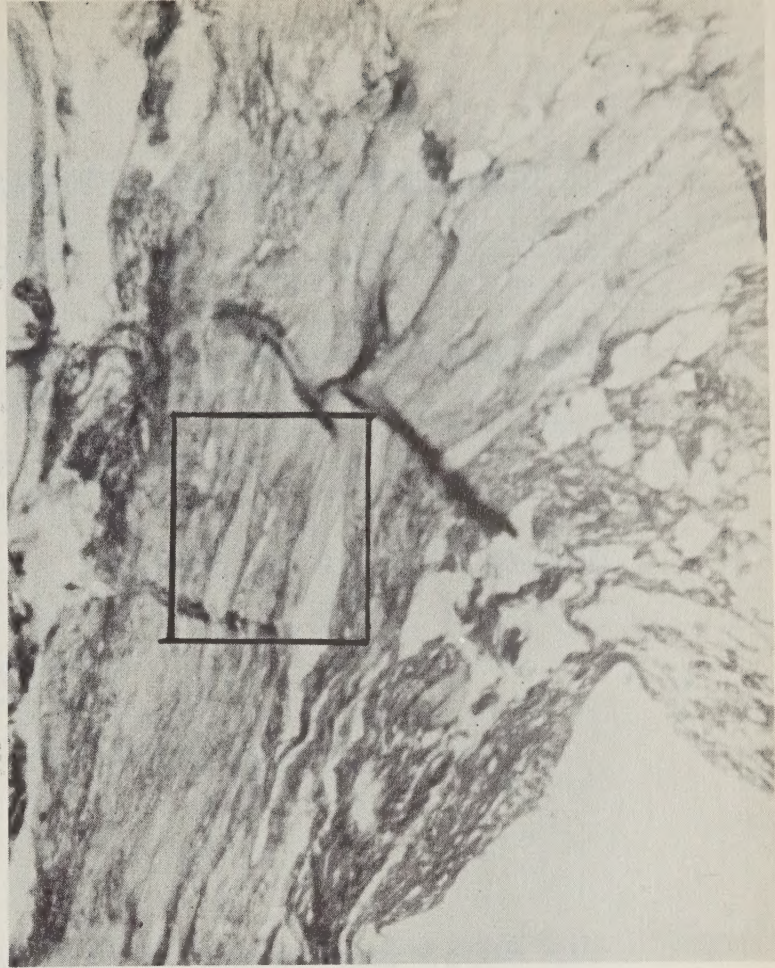
Fig 23







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27



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APPENDIX E

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Study of human dental pulp vessels by x-ray microarteriographic methods.

Grantee: Dr. R. L. deC. H. Saunders,
Dalhousie University.

Project No.: DR-51 Amount of Grant: \$1,400.

SUMMARY OF WORK

Experimental procedure: see Progress Report

Results: see Progress Report.

Conclusions:

1. The study of the developmental changes that occur in the intradental and peridental vascular nets should be continued, and steps have been taken to ensure a better supply of foetal material.
2. The injection techniques for demonstrating the vascular patterns of the adult dental pulp show improvement, and further series of adult teeth should be injected and examined under the new equipment. Whenever conditions permit it is intended to study the peridental tissues in a similar manner; post-mortem regulations make the latter procedure difficult.
3. The new microfocus X-ray equipment recently provided by the committee has, as was anticipated, led to markedly better resolution of both vascular and tissue details. Both microradiography (or high resolution contact radiography), and X-ray microscopy (or high resolution direct enlargement radiography) are now practised in this laboratory, and it is felt that these methods should be applied to both foetal and adult dental specimens in conjunction with freeze drying techniques, since pilot experiments have shown that this greatly improves the detail discernible.
4. The present state of these studies on the vascular architecture of the pulp, and in particular the arrangement and nature of the subodontoblastic plexus is such to support the procedure of non-infective pulp capping. Similarities and differences in pulp vascular patterns must receive further consideration. It is anticipated that such vascular studies will not only influence ideas regarding the growth and eruption of the tooth, but also pathological and clinical thought.

PROGRESS REPORT

Experimental Procedure

On receiving the teeth from the dental clinic they were plated on a 5" x 7" cardboard cassette and a scout film taken at the following factors KV 65 MA 100 Distance 40 ins. Time .25 secs., in order to determine the teeth most likely to receive adequate injection.

Thereafter the teeth selected were drilled occlusally until the pulp chamber was barely opened, with of course some tearing of the finer sub-odontoblastic vessels. The teeth were then placed in the rubber nipple of the modified injection apparatus, reduced pressure was obtained and held for 24 hours, at which point the tooth was removed, rinsed in water, and x-rayed on a dental film at KV 35 MA 20, Distance 14 ins., Time Range 4 - 5 secs. (anterior teeth) to approximately 3 minutes (heavy posterior teeth), to ascertain if the tooth was injected.

Successfully injected teeth were microradiographed on 2 x 2 in. Contrast Plate at factors of KV 35, MA 20, Distance 14 inches. Time range varied with the state of the tooth (see below). These teeth were then placed in 10% formalin for a week, following which they were placed in Versene pH 5. Sufficient decalcification had occurred after 3 weeks to permit manual slicing of the tooth parallel to its long axis; after which they were again x-rayed on the contrast plate with of course correspondingly shorter x-ray exposures. The teeth were then replaced in Versene and the decalcification, slicing, and x-raying procedures continued until further slicing would endanger the injected vessels within the pulp. The lattermost x-ray pictures showed the greatest definition and clarity of the injected vessels due to their exposure by the slicing and decalcification process just described.

Dissatisfaction with the percentage of teeth injected by the procedure previously reported, led to certain modifications of that technique. Firstly, an 18 inch glass tube, $\frac{1}{4}$ inch internal diameter, was bent into U-shaped fashion, and a 6 inch length of $\frac{1}{2}$ inch pressure tubing attached to the horizontal end. A number 1 rubber stopper was fitted over the vertical end and pushed down an inch. A $1\frac{1}{2}$ inch length of pliable latex tubing was then pushed over the exposed $1\frac{1}{2}$ inches of vertical tubing until it made contact with the rubber stopper where it was sealed with rubber cement. This served as the tooth nipple. Mercury was introduced into the U tube until a 3 inch column was obtained in each limb.

The crown of the drilled tooth was then inserted into the open end of the latex tubing until it rested against the free end of the glass of the U tube to obviate collapse of the soft latex tubing or tooth nipple by the reduced pressure. The tooth nipple around the crown was then pinched by fingers (digitally) to allow passage of air. The entire U tube was tipped until the mercury ran up to the tooth so expelling the air in the tube through the pinched opening. When a bead of mercury appeared at the nipple opening the latter was released and the tube returned to a vertical position. Adequate seal around the tooth crown was indicated by the maintenance of an altered level in the mercury column; a $\frac{3}{4}$ x 6 in. glass tube fitted over the tooth and rubber stopper served as a reservoir for the contrast medium.

Diluted contrast medium (20% Thorotrast having been found to give better results) was now placed in the reservoir until the tooth apex was covered by at least $\frac{1}{2}$ inch. Suction was then applied to the free end of the pressure tubing by rotary pump, until the U tube system was under a negative pressure equivalent to 120 mm of vacuum. The pressure tubing was then clamped and the unit removed to a stand for 24 hours. The levels of the mercury within the U tube were marked on the left hand tube before the suctioning and after, since they provided a useful index as to whether Thorotrast had entered the tooth, or air into the clamped end of the tube.

Since this dental injection apparatus could be handled as a unit, similar units were made so enabling an increased production of injected teeth. Six such units were set up.

The exposure of the pulp cavity vessels has also been modified. The cuspal eminences of the tooth were removed by judicious grinding with an unmounted dental wheel stone. The dentine thus exposed was then removed to within an estimated .5 mm of the pulp with a 557 Fisher burr and entrance to the chamber itself was made very carefully with a No. 1 round burr.

In the event that the occlusal surface of the tooth was destroyed by cavity the above procedure was followed with the exclusion of the grinding stone. On occasion iris scissors were used to nick the subodontoblastic plexus, but more recently the action of the burr was found to open these sufficiently.

Results:

The mercury proved superior to the sodium citrate method previously employed because it provided a definite vacuum seal which was in no way reduced by dissolved gases

escaping out of the solution into the vacuum.

During the last summer 48 teeth were injected by this procedure. With this procedure 75% to 80% successful injection was obtained with those teeth considered suitable for processing; suitability depended upon the degree of caries and the presence or absence of pulp cavity involvement.

Various technical problems connected with the new x-ray equipment and the microradiographic rendition of tissue and vascular detail were studied last summer (1955) in Britain under a Nuffield Travel Grant. Equipment tests were made on various types of fine focus x-ray tubes or x-ray microscopes at Hilger's X-ray Research and Development Laboratory at Camberwell, Mr. Raymond Ely's (Foster Transformers) Laboratory in Wimbledon, and Professor Coslett's and Dr. Nixon's Laboratory at the Cavendish Physics Laboratory in Cambridge. Comparative tests on injected material were made on these instruments, all of which has led to an improvement in technique with consequently better resolution of tissue and vascular detail.

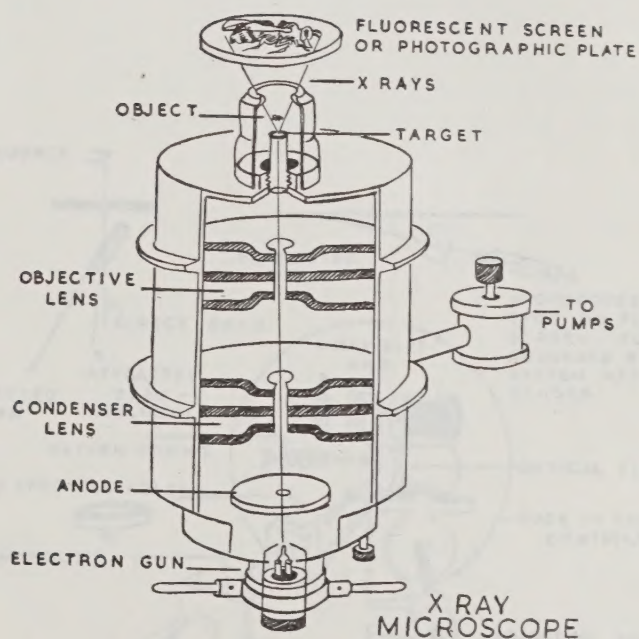
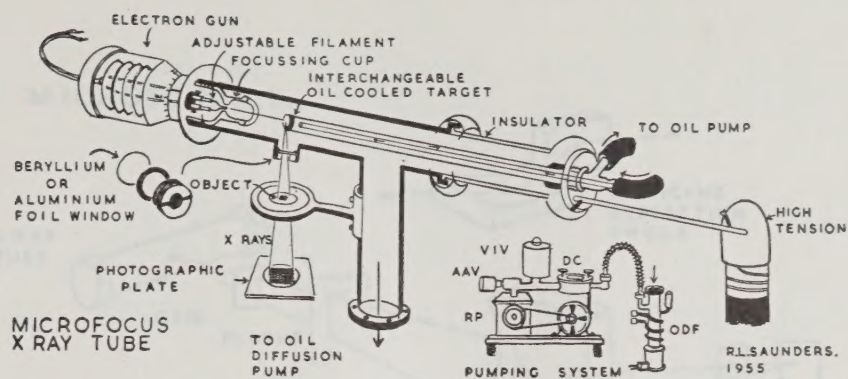
Tube focussing studies were made using grids, and a fine source of x-rays or focal spot of 5 microns was achieved, and confirmed by Prof. W. Ehrenberg, Birbeck College, London University, who designed the prototype of the tube we are now using. As a result the resolution is better and our microradiographic pictures may be said to compare favourably with those produced elsewhere. It is hoped therefore that the N.R.C. Dental Committee will assist us in following a long range study of dental microradiography.

The useful range of wavelengths for clinical radiography is commonly stated to be approximately .5 to .125 Angstroms, but with the demountable tube now in use we are able to take advantage of the fact that the lower the atomic number of the target material the longer the wavelength of the characteristic radiation. These longer (softer) waves provide better tissue contrast. When the wavelength is to be altered the target material in the x-ray tube is changed. To reduce the absorption of the softer rays an experimental camera was built in which pictures of tissue can be taken in vacuo. It should be understood that all operations however have to be undertaken in darkness.

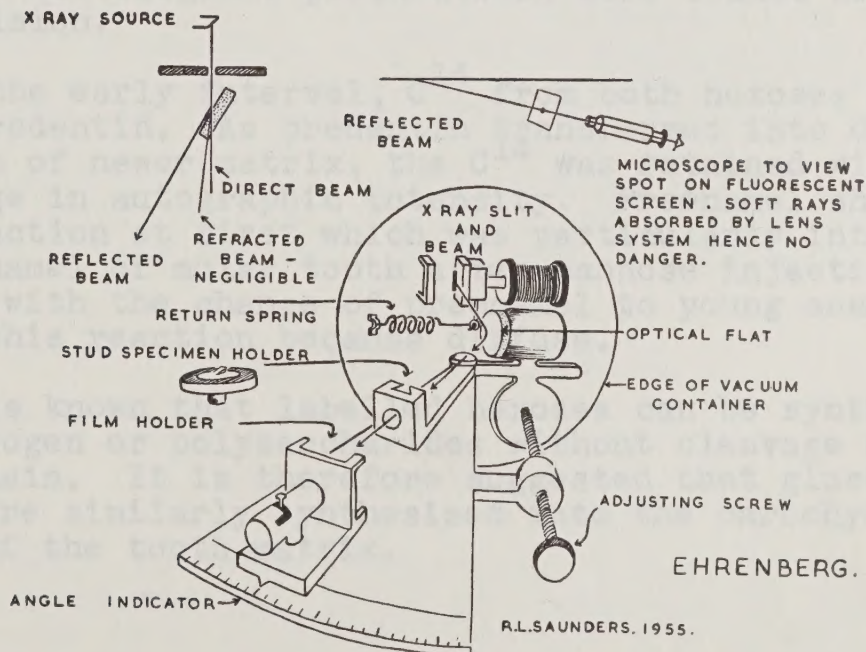
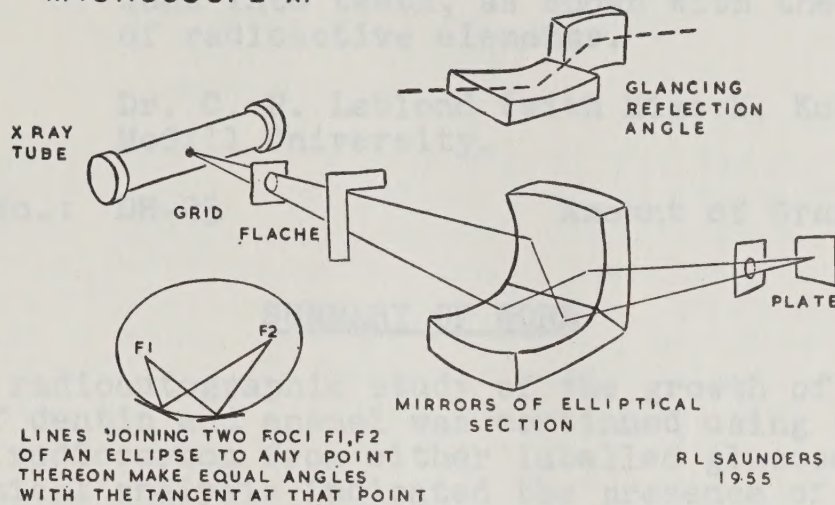
Pilot experiments were made with freeze-drying techniques, and since this has greatly improved the detail discernible a freeze drying unit is requested.

Illustrations of the various types of equipment either examined or used during the last summer in Britain and France are attached hereto. These drawings were made by the applicant in preparation for an article on microradiographic and x-ray microscope techniques, wherein due acknowledgement will be made to the kind assistance of Professors Coslett, Cauchois, Ehrenberg, Dr. Nixon, Messrs. Hilger and Watts, and Mr. Ely of Foster Transformers Ltd.

A paper entitled "Etudes microarteriographiques des vaisseaux pulpaire de la dent humaine" that dealt with the technical procedures and certain particulars of the foetal and adult human intradental and peridental vessels as revealed by our microarteriographic methods, was presented to the VIe Congres Federatif International d'Anatomie at Paris in July 1955, with an opening acknowledgement to the assistance rendered by the Dental Committee of the National Research Council. In addition a technical paper has been completed and is now being forwarded to the press, and is being followed by another on developmental aspects.



MICROSCOPE X.



APPENDIX F

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.

Grantee: Dr. C. P. Leblond (with Miss Y. Kumamoto)
McGill University.

Project No.: DR-23

Amount of Grant: \$3,350.

SUMMARY OF WORK

The radioautographic study of the growth of the organic matrix of dentin and enamel was continued using the incorporation of radiocarbon from either labelled glucose or mannose. Histochemical analysis indicated the presence of polysaccharides, while chemically, simple sugars were separated from hydrolyzed human dentin (report, March, 1954) and presently, also from rat dentin. It was hoped by this method, to elucidate some of the mechanisms operating during matrix formation.

Three day old rats injected with either labelled glucose or mannose-1- C^{14} were sacrificed 1 1/2-3, 24, and 72 hours after injection. The jaws were removed and fixed in an alcohol-formaldehyde solution. For radioautography, stained and unstained histological preparations were coated with Kodak NTB3 emulsion.

At the early interval, C^{14} from both hexoses was found in the predentin. As predentin transformed into dentin with formation of newer matrix, the C^{14} was retained with hardly any change in autographic intensity. Preenamel showed a definite reaction at first which was particularly intense in the preenamel of molar tooth after mannose injection. Concomitant with the change of preenamel to young enamel during growth, this reaction became diffuse.

It is known that labelled hexoses can be synthesized into glycogen or polysaccharides without cleavage of the carbon chain. It is therefore suggested that glucose and mannose are similarly synthesized into the carbohydrate components of the tooth matrix.

PROGRESS REPORT

Radioautographic localization of radiocarbon in the teeth after administration of labelled glucose and mannose: and a chromatographic identification of carbohydrates of rat dentin

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The investigation of the growth of rat teeth, and more particularly of dentin was continued using the radioautographic method. Previously, mineral deposition was visualized by Ca^{45} or P^{32} (Kumamoto), while the formation of matrix was seen with C^{14} -bicarbonate (Greulich). There was a rapid incorporation of these materials into the portions of teeth undergoing formation. Histochemical observation, especially by means of the PA-Schiff technique indicated that carbohydrates of large molecular weight were present in dentin. Chemically, it was shown that, after hydrolysis of human dentin (report, March, 1954), several hexoses and one pentose could be recovered. These data prompted us to investigate tooth growth utilizing labelled hexoses, in the hope that their incorporation would throw light on the problem of matrix formation.

A radioautographic study commenced with radioglucose administered to newborn rats (report, January, 1955) was repeated, this time injecting the labelled glucose solution to 3 day old rats. The results were identical with those obtained with newborn rats. In addition, a radioautographic survey of the incorporation and fate of C^{14} after radiomannose injection was studied. Generally, the autographic distribution of radiocarbon was similar to that after glucose administration.

The chromatographic identification of dentinal carbohydrates was continued, this time utilizing dentin from rats. The method for sugar analysis developed in this department was used.

Materials and Methods

Radioautographic study.

Radioglucose

Three black and white hooded rats, 3 days of age (6 grams) were given subcutaneously, 30 μc of a solution of generally labelled glucose in a volume of 0.06 cc. A # 26 hypodermic needle was inserted in the dorsal neck region and passed caudally

through the hypodermis and the labelled glucose was deposited over the rump. The rats were killed after chloroform anesthesia at 1 1/2, 24 and 72 hours later. Various organs were fixed without delay in Orth and also in alcohol-formaldehyde solution. Stained and unstained histological sections coated with 1% celloidin were coated with Kodak NTB3 emulsion dried and exposed for 7 months. After exposure, they were processed by the usual autographic procedure.

Radiomannose

Three albino rats, 3 days old (9 grams) were utilized in the experiment. They received 23 μ c of mannose-1-C¹⁴ in a volume of 0.07 cc in the same manner as in the above experiment. They were killed after ether anesthesia at 3, 24, and 72 hours intervals. The tissues were fixed in three different fixing solutions; namely alcohol-formaldehyde, Orth, and Bouin. The autographic procedure was identical to that of glucose. Exposure time was four months in this case.

Carbohydrate of rat dentin

Adult albino rat incisors were extracted, freed of as much pulp matter and alveolar bone and then decalcified in 0.2% HCl solution. As they became decalcified, they were freed of enamel and the pulp cavity opened and any remaining pulp removed. Decalcification was continued with removal of the HCl solution until the dentin became pliable and soft. The sample was then washed free of chloride, which was considered to indicate that it was free of HCl. Extraction of the carbohydrates was carried out by the method developed in this department by Glegg and Eidinger. The method can be summarized in the following scheme.

Extraction of tissue with 0.5 N sodium hydroxide
(4 days in cold)

Filter through gauze

alkaline extract - neutralize with
acetic acid

reduce volume of extract

add alcohol (1% KAc, 1%HOAc)

Fraction I
63% conc. alcohol

Supernatant
↓
add alcohol (1% KAc, 1%HOAc)

Fraction II
84% alcohol conc.

Fractions I and II were washed with absolute alcohol and ether and dried at room temperature.

The precipitates were then hydrolyzed. Hydrolysis was carried on in an aqueous medium with the resin, Permutit Q, a sulphonated polystyrene resin in the hydrogen or cationic form. The tube was sealed and left in an oven at 100°C for 2 days, after which the fluid was concentrated in a vacuum oven at 37°C. The carbohydrates were then separated and identified by paper chromatography using pyridine-butanol-water solvent. After three developments (ascending) the paper was sprayed with aniline hydrogen oxalate reagent and the colour brought out by putting the paper in an oven at 100°C for several minutes.

Radioautographic Results

Radioglucose

The autographic results of the present experiment were identical to those obtained after injection of radioglucose to newborn rats. Radiocarbon was found in the predentin at the earliest interval, which later became the dentin when new layers of matrix formed on it. By the third day, the reaction was seen well within the dentin (fig. 1). The reaction over the enamel was diffuse with slightly greater amounts of radioactivity in the areas of preenamel, especially at the early interval.

Radiomannose

With mannose injected tissues, a definite increase in the overall reaction of the tooth and bone-cartilage matrix was noted from the first to the second interval. By the third interval, however, the reaction in the cartilage matrix decreased to the extent that it was lighter than that of the first interval. In spite of this fluctuation in the reaction of cartilage matrix, that of the dentin and bone appeared undiminished. The soft tissues, in contrast, did not react in this manner; they were most intense at the first interval, and decreased gradually thereafter.

Three hours after injection of mannose-1-C¹⁴ (figs. 2 & 5), a pronounced radioactivity was found in the predentin, and only a light reaction was found over the rest of the dentin. (The cells of the pulp reacted lightly, with the odontoblasts reacting slightly more than the rest.) The preenamel at this interval showed an intense, well limited deposition of C¹⁴, the other parts of the enamel reacting only lightly. (Of the parts of the enamel organ, the apical

portion of the ameloblasts and the stratum intermedium reacted more than the outer enamel epithelium and the stellate reticulum which were very light radioautographically.)

¹⁴C Twenty-four hours after injection (figs. 3 & 6), the formerly found in the predentin was now in the dentin adjacent to the predentin. The enamel showed a general reaction maximal in the region midway in its thickness between the dentino-enamel junction and the forming enamel, presumably in the region called "young enamel". The cells of the tooth germ are only lightly reacting. Most clearly noticeable in the molar tooth (figs. 5 & 6), the sharpness of delineation of the autographic reaction observed at the first time interval was decreased.

At the third interval (figs. 4 & 7), the radiocarbon was well within the dentin, still in the form of a band; the intensity of reaction appeared little changed during the experimental period. The other parts of the dentin contained a lighter diffuse reaction. The enamel lost its intense band of reaction (fig. 7), and in its place was a diffuse reaction decreasing from the older parts of the enamel. The cells of the developing tooth showed only a very weak reaction at this interval.

Carbohydrates of rat dentin

By the chromatographic method, the following sugars were recognized: glucuronic acid and its lactone, galactose, glucose, mannose, and traces of fucose, xylose and arabinose (fig. 8).

Discussion

Although radiocarbon from glucose and mannose was found incorporated soon after injection in all tissues of the body, some comment on the similarities and dissimilarities of the autographic reaction of soft and hard tissues may be in order.

With most soft tissues, the passing of time resulted in lowering of the autographic intensity (liver, lung, pancreas). Much of this decrease in autographic intensity may be ascribed to either the loss of the radiocarbon from the tissue by catabolism or to a dilution of the radiocarbon present due to the appearance of new, unlabelled material.

In the case of the mucus of the sublingual glands, the intense reaction obtained soon after injection was no longer observed 24 hours later and thereafter. Thus, radioactive mucus is formed soon after injection and eliminated; but the mucus formed subsequently does not show radioactivity. The mucus of the gastrointestinal tract behaved in the same manner, especially that of the goblet cells.

In the hard tissues - teeth and bones - growth involved transformation of an organic precursor of matrix (predentin, preenamel, preossein) into the organic matrix proper, with a concomitant change in histological staining properties. The C^{14} from either glucose or mannose was taken up into the matrix precursor and later remained within the more mature matrix arising from the precursor. Notwithstanding the similarity in the transformation of pre to mature matrix, autographic reaction from such tissues can be divided into two categories. In dentin the band reaction due to radiocarbon uptake in predentin is retained without loss in the course of its transformation.

The observation that this band appears farther and farther from the pulp with time demonstrates the incremental growth of dentin. In contrast, preenamel showed an immediate uptake of radiocarbon, but, as the reaction appeared in young and later in maturing enamel, a progressive decrease in its intensity was seen. This behaviour was similar to that observed after radiosulphur administration in the rat enamel (Belanger). The loss is thought to be due mostly to the dispersion of the organic matrix of the enamel during mineralization.

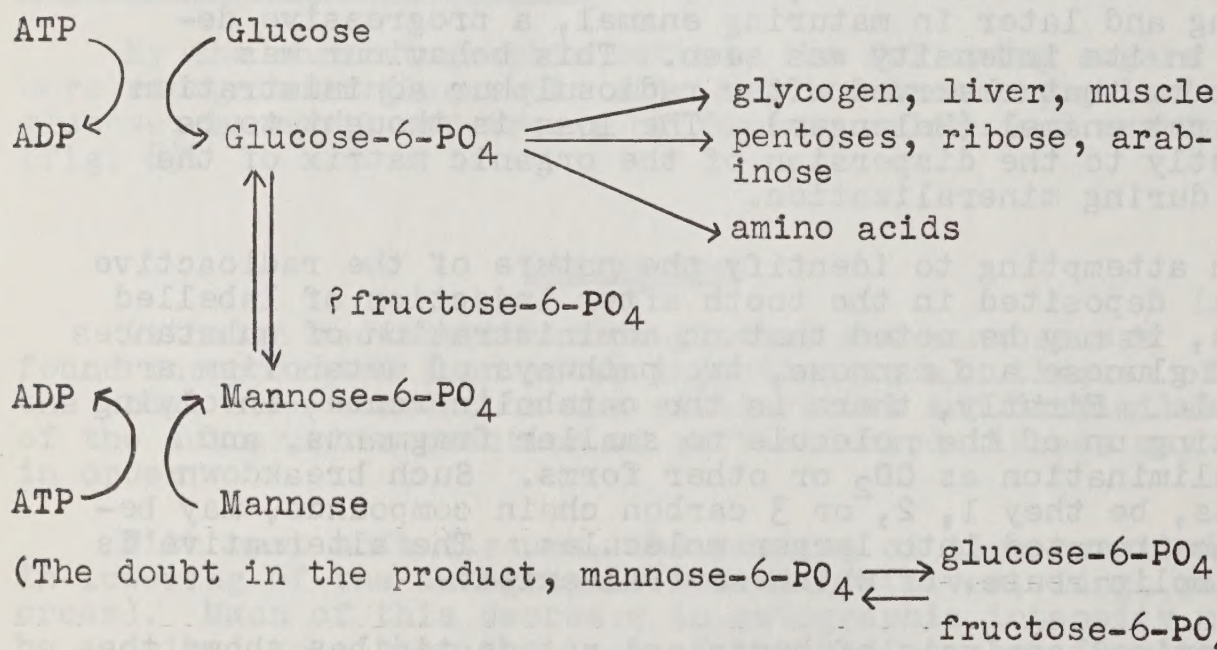
In attempting to identify the nature of the radioactive material deposited in the tooth after injection of labelled hexoses, it may be noted that on administration of substances such as glucose and mannose, two pathways of metabolism are available. Firstly, there is the catabolic route, involving a breaking up of the molecule to smaller fragments, and their elimination as CO_2 or other forms. Such breakdown products, be they 1, 2, or 3 carbon chain compounds, may become incorporated into larger molecules. The alternative is the anabolic route, of which several exist.

Chemical analysis of human and rat dentin has shown the presence of sugars, thus confirming the positive reaction of PA-Schiff method to indicate the presence of carbohydrates. Furthermore, metachromasia of dentin with toluidine blue indicate the presence of mucopolysaccharides. Moreover,

Belanger has demonstrated the presence of sulfur-containing substances in the tooth, and has identified them tentatively as being mucopolysaccharides.

It is known that labelled hexoses may be incorporated into glycogen or polysaccharides without alteration of the carbon chain sequence. Thus, Cook and Lorber, investigating the metabolism of glucose-1- C^{14} and mannose-1- C^{14} in liver and muscle glycogen, found 80-90% of the activity isolated in the glycogen resided in carbon number 1. They showed that both hexoses behaved in the same manner, due to a conversion of mannose to glucose which does not involve carbon chain cleavage. Chung and Nickerson concluded from their work with polysaccharide synthesis in growing yeasts, that an enzymatic interconversion of glucose-1-phosphate and mannose-1-phosphate exists through a hexose-6-phosphate step. Furthermore, Roseman et al found that the glucuronic acid portion of hyaluronic acid produced by a strain of Group A streptococcus arises from glucose without splitting of the carbon chain. Further evidence indicated (Roseman) that a similar mechanism obtains with respect to the formation of glucosamine.

If mannose and glucose are similarly metabolized in the formation of dentinal matrix, the first step in the incorporation of hexoses into organs or organisms is by phosphorylation as in the scheme (Fruton and Simmonds):



(The doubt in the product, mannose-6-PO₄ $\rightleftharpoons$ glucose-6-PO₄ $\rightleftharpoons$ fructose-6-PO₄ is due to the fact that there has been phosphohexoisomerase in the enzyme preparation, Slein). Thus, it is quite probable that the odontoblasts and ameloblasts, which synthesize the organic matrix of dentin and enamel respectively, are

capable of transforming hexoses into larger carbohydrate complexes without scission of the 6 carbon chain.

While the breakdown products of labelled glucose and mannose (e.g. CO_2) may complicate the picture, as well as other interactions, e.g. at the cross roads of protein and carbohydrate metabolism, it can be pointed out that autographic patterns such as that observed in the developing enamel are not observed after injection of labelled CO_2 . Therefore, it appears reasonable to conclude that a direct incorporation of an hexose into a complex carbohydrate has taken place.

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- Fig. 1 Coated autograph of a hematoxylin and eosin preparation of a molar cusp of a 3 day old rat injected with radioglucose, and sacrificed three days later. x 215. Note the reaction ($\uparrow$) in the dentin (D), while the enamel (E) shows a diffuse reaction on the side of the dentin. An artificial space separates the enamel and dentin. (P) = pulp.
- Figs. 2 - 7 Coated radioautographs of teeth of rats which were injected with mannose-1-C¹⁴ at 3 days of age and sacrificed after various time intervals. E = enamel; D = dentin; P = pulp; $\uparrow$ = autographic reaction in the form of a band.
- Figs. 2,3,4 Incisor teeth, hematoxylin and eosin stained. x 215.
- Figs. 5,6,7 Molar teeth, unstained preparation. x 21.5
- Figs. 2 & 5 3 hours after injection.
- Figs. 3 & 6 24 hours after injection.
- Figs. 4 & 7 72 hours after injection.

The autographic reaction is present in the predentin at the first interval (figs. 2 and 5) which later is found covered with a layer of newly formed dentin (figs. 3 and 6). At the third interval, the radiocarbon is laid well within the dentin (figs. 4 and 7). The intensity of the reaction appears undiminished during the experimental period.

- Fig. 8. Chromatogram of rat dentin.

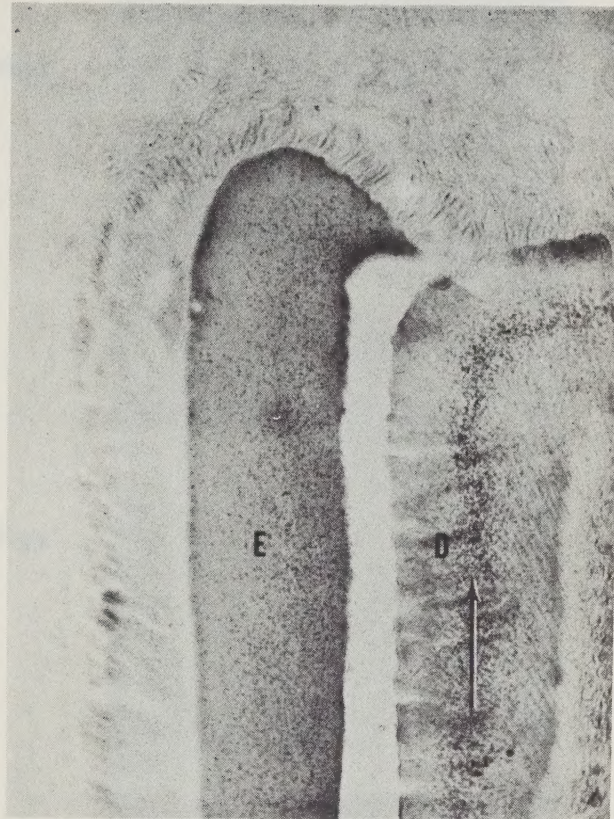


FIG. 1

Fig. 2

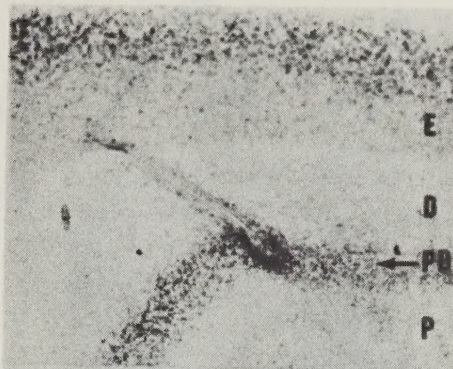


Fig. 3

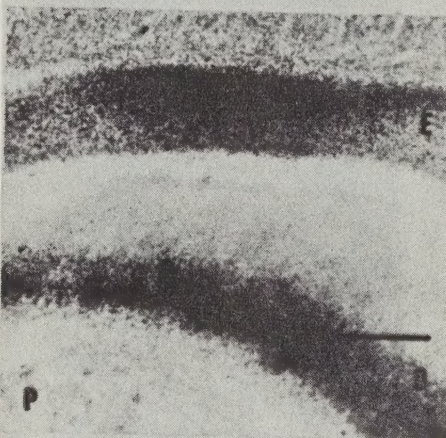
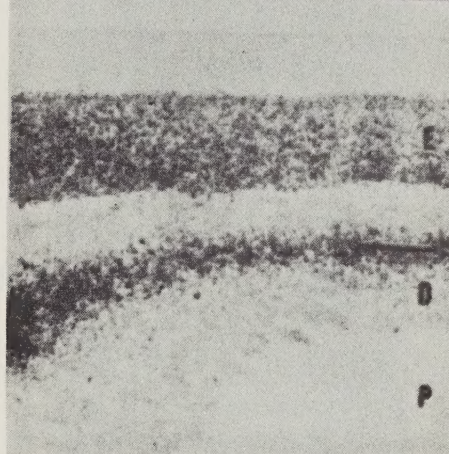


Fig. 4



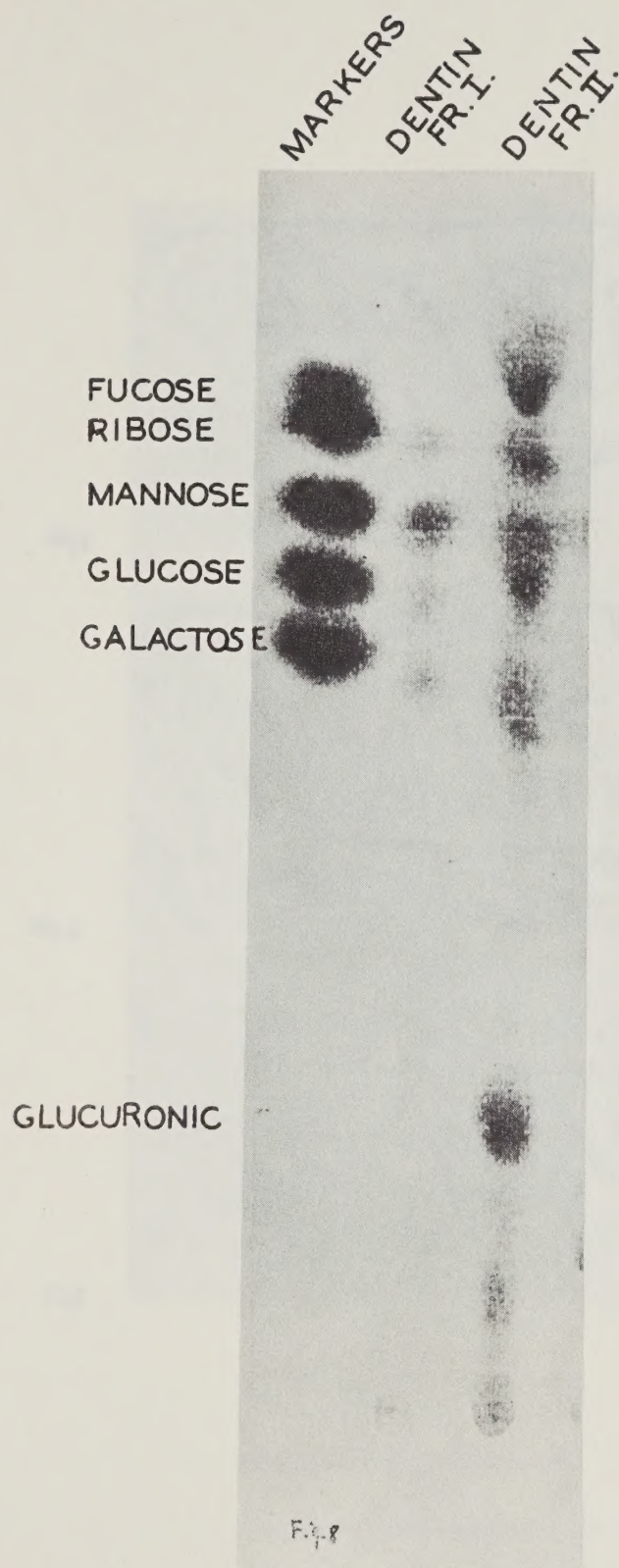




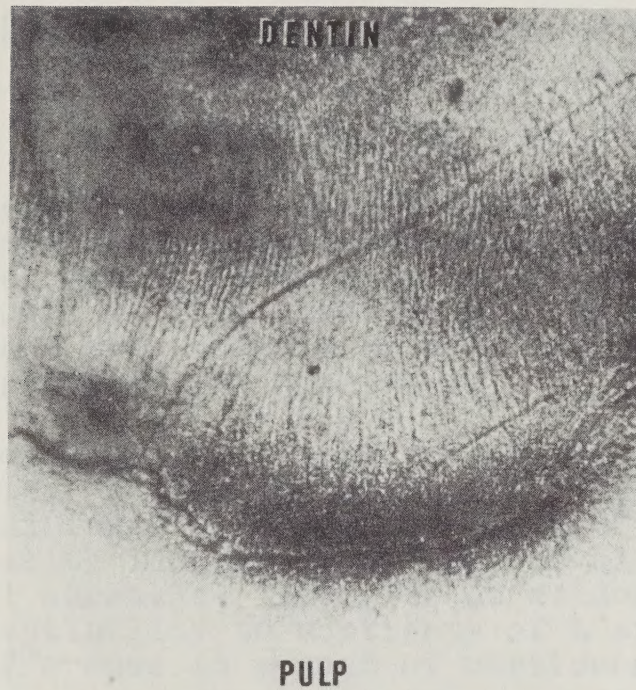
Fig. 5



Fig. 6



Fig. 7



Radioautograph of dentin obtained one month after injection
of Ca 45 into a 6 month old rat. X 500 app^x

The dark band located in the portion of dentin close
to predentin is interpreted as indicating that some deposition of
new dentin is taking place even in such a relatively old animal.

APPENDIX G

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Pathogenesis of cortisone-induced cleft palate in mice.

Grantee: Dr. F. C. Fraser,
McGill University.

Project No.: DR-60

Amount of Grant: \$1,850.

SUMMARY OF WORK

The frequency of cleft palate in the offspring of pregnant mice treated with cortisone depends partly on the maternal and foetal genotypes (Fraser et al, J. Cell. Comp. Physiol. 43, Supp. 1: 237, 1954; Kalter, Genetics 39: 185, 1954). To extend the analysis of this teratologic model, the following experiments have been done:

1. It has been shown that cortisone given to pregnant strain C57BL mice reaches the embryos in amounts great enough to cause a significant decrease in volume of the foetal adrenal. Preliminary results indicate that this is also the case for strain A foetal adrenals. There is no evidence that the difference in susceptibility to cortisone of A and C57BL strains is due to a difference in amount of cortisone reaching the foetus.
2. Observations on palate closure time have shown that strain A embryonic palates close at a later chronological age and morphological stage of development than strain C57BL palates. Present results indicate that A x C57BL and C57BL x A palates close at an intermediate morphological stage of development between the parent strains, while chronologically C57BL x A palates appear to start closing even earlier than C57BL palates. These results are consistent with the idea that embryos in which the palate closes relatively late are more susceptible to the cleft palate producing effects of cortisone than those in which the palate closes early.
3. Since light females are more susceptible to cortisone than heavy females, the palate closure pattern of embryos from light versus heavy females is also being studied, but the data are not yet ready for analysis.
4. Material is being collected for a comparison of morphological development of embryos with and without cleft palates in cortisone-treated litters, to see if the relatively retarded

ones are the ones that get cleft palates.

5. The effect of introducing needles, cortisone or physiological saline directly into the pregnant uterus is being studied. So far cleft palates have been produced by amniotic sac puncture, and by cortisone vehicle or physiological saline injected into the uterus. This discovery has provided an experimental demonstration of a new mechanism through which congenital cleft palate may occur.

SUMMARY OF WORK

1. The frequency of cleft palate in the offspring of pregnant mice treated with cortisone depends partly on the maternal and fetal genotypes (Fraser et al., J. Cell. Comp. Physiol. 43, Suppl. 1: 237, 1954; Kletter, Genetics 93: 185, 1954). To extend the analysis of this teratologic model, the following experiments have been done:
 1. It has been shown that cortisone given to pregnant strain C57BL mice reaches the embryos in amounts great enough to cause a significant decrease in volume of the foetal adrenal. Preliminary results indicate that this is also the case for strain A foetal adrenals. There is no evidence that the difference in susceptibility to cortisone of A and C57BL strains is due to a difference in amount of cortisone reaching the foetus.
 2. Observations on palate closure time have shown that strain A embryonic palates close at a later chronological age and morphological stage of development than strain C57BL palates. Present results indicate that A x C57BL and C57BL x A palates close at an intermediate morphological stage of development between the parent strains, while chronologically C57BL x A palates appear to start closing even earlier than C57BL palates. These results are consistent with the idea that embryos in which the palate closes relatively late are more susceptible to the cleft palate producing effects of cortisone than those in which the palate closes early.
 3. Since light females are more susceptible to cortisone than heavy females, the palate closure pattern of embryos from light versus heavy females is also being studied, but the data are not yet ready for analysis.
 4. Material is being collected for a comparison of morphological development of embryos with and without cleft palates in cortisone-treated litters, to see if the relatively retarded

PROGRESS REPORT

It has been shown (Fraser et al, 1954) that there is a marked difference in frequency of cleft palate in the offspring of cortisone-treated female mice of strain A/Jax (henceforth called 'A') and strain C57BL (called 'B'). The frequency of cortisone-induced cleft palate must therefore be genetically determined. There is also a reciprocal cross difference in cleft palate frequency in the offspring of crosses between the two strains (Kalter '54). When 2.5 mg of cortisone was given to mothers on each of 4 successive days starting at gestation day 11 the results were as follows:

<u>Cross</u>		<u>% Cleft Palate in Offspring</u>
1.	A x A	100
2.	B x B	18
3.	A x B	43
4.	B x A	4

A comparison of crosses 1 and 3 or 2 and 4 shows that in the same uterine environment genetically different embryos have different frequencies of cleft palates. The genotype of the embryo must therefore be important in determining whether or not it gets a cleft palate.

The reciprocal cross difference shows that the embryo's maternal environment is also important. These reciprocal cross differences could be due to a strain difference in the mother's reaction to cortisone, or to a strain difference in placental permeability causing a difference in the amount of cortisone reaching the foetus. It could also be due to a difference in the reaction of the foetus to cortisone, determined by the nature of the maternal-foetal relationship.

The purpose of the present experiments is to continue the analysis of these maternal-foetal interactions, and thus come to understand more about the etiology of congenital cleft palate. At present four approaches are being made to the problem.

1. Observations on Foetal Adrenals

Foetal adrenals in the rat have been shown to decrease in volume when cortisone is injected into the mother (Davis and Plotz, 1954). Thus it appeared worthwhile to study the adrenal histology of embryos from A and B mothers which had received cortisone, and to compare these with controls. If there was a strain difference in the amount of cortisone reaching the

embryo it was hoped that the foetal adrenals of B (the resistant strain) might show a much lower response to the treatment than those of strain A.

Methods. B mothers and A mothers were treated with 2.5 mg cortisone given on four successive days starting 11 days after observation of a vaginal plug (day 11). They were sacrificed on day 15, the embryos being fixed in Bouins. After 2 to 4 days in Bouins they were transferred to and stored in 70% ethyl alcohol. The two kidneys plus attached adrenals were carefully dissected away from the embryo, embedded, sectioned, and stained with H. and E.

Outline drawings of every fifth adrenal section were made using a microprojector. The area of each drawing was then measured using a planimeter and the volume of the adrenal estimated by the method described by Josimovitch et al (1954).

Results. The results obtained so far are summarized in table 1.

Table 1

Volume (mm³) of day 15 foetus adrenals from mothers treated with 2.5 mg/day, x 4 beginning on day 11, and of control adrenals.

Strain	Cortisone treated		Control	
	Mother	Adrenal vol.	Mother	Adrenal vol.
B	618-1	0.0273	619-1	0.0560
		0.0282		0.0499
	618-2	0.0400	619-4	0.0559
		0.0387		0.0651
	480-1	0.0268	619-3	0.0550
	480-2	0.0211		0.0605
	Mean	0.0287 $\pm$ 0.0039		0.0571 $\pm$ 0.0022
A	569-4	0.0325	599-1	0.0408
		0.0323		0.0375
			599-2	0.0422
				0.0404
			599-3	0.0438
				0.0588
			100-1	0.0475
	Mean	0.0324		0.0451 $\pm$ 0.0029

t test B treated vs B control - t = 6.399, p $\ll$ 0.01

A control vs B control - t = 3.275, p < 0.05

Both adrenals were measured from each embryo, except in 480-2 and 480-1 where one adrenal was measured in each case. The mean adrenal volume of strain B treated embryos was $0.0287 \pm 0.039 \text{ mm}^3$ while that of B control embryos was 0.0571 ± 0.0022 . A t test for the difference of these two means gives $t = 6.399$ with a $P < .01$. The foetal adrenals from treated B mothers are therefore significantly smaller than the control foetal adrenals.

Only one pair of adrenals from treated A have been measured so far, so that a t test cannot be made to compare the treated A with the control. The mean volume of 0.0324 for the A treated embryo is lower than the mean control volume of $0.0451 \pm 0.0029 \text{ mm}^3$ (for strain A). More embryos have been collected to enlarge the present observations.

Conclusions. The present results have shown that cortisone reaches the B embryos and probably the A embryos in large enough amounts to cause partial atrophy of the foetal adrenal. Microscopic examination of sections also shows that the cells of treated adrenals are smaller than those of the control. In treated adrenal sections the nuclei appear more crowded together than in the control. Thus these preliminary results have shown that the treated B strain (resistant) foetuses receive active amounts of cortisone as do the A strain. There is, therefore, no evidence that the susceptible A strain and the resistant B strain foetuses are receiving different amounts of cortisone from the mother.

2. Observations on time of palate closure.

Previous work from this laboratory (Walker and Fraser, 1956) has shown that strain A embryonic palates close earlier than strain B palates. A study is now being made of the time of palate closure in A x B and B x A hybrids. At the same time further data are being gathered on the parent strains to check Walker's results.

Methods. For this purpose pregnant mothers are killed at two-hour intervals during the fourteenth and early part of the fifteenth day of gestation. The entire uterus is removed and fixed in Bouins for 2 to 4 days then stored in 70% ethyl alcohol. The embryos are then dissected out of the uterus and examined. A standard series of observations are made on the following morphological features: degree of finger webbing and hair follicle development, the appearance of the eyelids and the pinnae. Each feature is assigned a numerical value and the sum of these gives a figure named the morphological rating. This is a way of stating the stage of morphological

development, or developmental "age" of a particular embryo. The palate is then examined and given a numerical rating as follows: at stage 1 the palate is completely open, with the shelves hanging in the vertical plane on either side of the tongue, 2 shows early shelf movement, 3 shows one shelf in the horizontal plane, above the tongue, 4 both shelves are up, 5 the shelves are touching or fused at one point, at 6 they are partly fused, and at 7 the palate is closed.

Results: The chronological stage of the embryos was plotted against palate closure. It was found (table 2) that palate closure was from 14 days 8 hours (14/8) to 15/4 for the B strain, while for the A strain the time of palate closure was later and occurred between 14/18 and 15/8. The A x B hybrid embryo palates closed between 14/16 and 15/6, while the B x A palates closed between 14/0 and 14/20. The A x B palates thus close at about the same time as the A's, while the B x A palates start closing somewhat earlier than the B palates. These results indicate that the time of palate closure appears to be earliest in the most resistant cross B x A, while it is later in the susceptible A x A. Perhaps the strain and reciprocal cross differences in cortisone-induced cleft palate are at least partly a reflection of the normal pattern of palate closure.

Table 2

Cross	% Cleft Palate	Duration of Palate Closure	
		from days/hrs.	to days/hrs.
B x B	18	14/8	15/4
A x A	100	14/18	15/8
A x B	43	14/16	15/6
B x A	4	14/0	14/20

Tables 3, 4, 5, and 6 show the pattern of palate closure plotted against morphological rating. Comparing the A strain (table 3) with the B strain (table 6) it is evident that the B strain palate starts to close at an earlier stage of morphological development (stage 4, table 6) than the A strain (stage 8, table 3). At present the results with A x B and B x A (table 5) show that the

hybrids close at an intermediate period of morphological development between the 2 parent strains. B x A palates close earlier than the A strain and, in fact, appear to have a pattern very close to that of the B strain. There is also a slight indication that B x A palates close earlier than A x B.

Conclusions. These results are consistent with the idea that embryos in which the palate closes early are more resistant to the cleft palate producing effects of cortisone than those in which the palate closes later.

3. Palate closure in light versus heavy females.

Previous work (Kalter, 1955) has shown that the cleft palate incidence in the offspring of cortisone-treated backcross females which are young and light in weight is significantly higher than in the offspring of old heavy females (table 7).

Table 7

Light versus heavy females. Incidence of cleft palate in progeny of pregnant mice treated with 4 x 2.5 mg cortisone begun on day 11. All first parity (Kalter, '55).

<u>BA x A</u>	<u>% cleft palate</u>
20 lightest	67.8
20 heaviest	43.3

If the conclusion reached in the previous section is true, then light mothers should have embryos with later palate closure than heavy mothers. To test this, F1 females of the crosses A x B and B x A are being raised. These are backcrossed to A males either at puberty when they are light or after 4 months of age when they are heavier. The female weights are recorded on the day a vaginal plug is observed. Several litters have been collected and examined, but not enough to show any trends at present.

4. Morphological development of embryos with and without cleft palates

A problem about which we are almost completely ignorant is the question of why some embryos in a given uterus get cleft palates following a given stimulus and others do not. Perhaps it is because the relatively retarded embryos are

Tables 3, 4, 5, and 6 -- Relation of
palate closure to developmental age
(morphological rating^m) of embryo in
strains A/Jax ("A") and C57BL ("B") and
the F1 hybrids.

A x A		F1 Hybrids	
20 lightest	67.8	20 lightest	67.8
20 heaviest	43.3	20 heaviest	43.3

If the correlation reached in the previous section is true, then light mothers should have embryos with later palate closure than heavy mothers. To test this, 71 females of the crosses A x B and B x A are being raised. These are backcrossed to A males either at puberty when they are light or after 4 months of age when they are heavier. The female weights are recorded on the day a vaginal ring is observed. Several litters have been collected and examined, but not enough to show any trends at present.

4. Morphological development of embryo with and without cleft palate. A problem about which we are almost completely ignorant is the question of why some embryos in a given strain get cleft palates following a given stimulus and others do not. Perhaps it is because the relatively retarded embryos are

Table 3

A

Palate Stage

-3 11
-2
-1
0
1
2
3
4
5
6
7

Table 4

A x B

Palate Stage

-3
-2
-1
0
1
2
3
4
5
6
7

Table 5

B x A

Palate Stage

1
2
3
4
5
6
7

Table 6

B

Palate Stage

1
2
3
4
5
6
7

Morphological Rating

-3 11
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

1
2
3
4
5
6
7

most susceptible to teratogenic insults. To test this, an attempt will be made to discover, within litters from cortisone-treated females, whether embryos with cleft palates differ from non-affected litter mates as to morphological rating. For this purpose F1 females of the A x B and B x A cross are being raised. They will be mated with A males, treated with cortisone in the standard manner and sacrificed at day 15.

5. Supplementary studies on the causes of cleft palate

a) Amniotic sac puncture. Preliminary work (Walker, unpublished) showed that the introduction of a hypodermic needle (size 26) into the amnion, on day 13 of gestation, in some cases caused cleft palate in the embryo. Further work has substantiated these results.

The method was to etherize a pregnant female on day 13 of gestation, and to make an abdominal incision. The needle was then inserted into the amnion of each embryo in turn in one uterine horn. The embryos in the other horn were counted to act as control. The mother was then sewn up and was sacrificed on day 18 just before term. The results can be tabulated as follows:

No. ♀ operated	No. litters aborted	No. litters examined	No. cleft palates	No. cleft palates
			Treated horns	Control horns
14	6	8	10/17	0/15

Thus 10/17 or 59% of punctured embryos, which survived, had cleft palates. These cleft palates appear to result from a loss of amniotic fluid which constricts the embryo, pushing the head down on the chest and thus keeping the tongue forced between the palatine shelves which cannot then fuse.

b) Intra-uterine injection. In an attempt to get cortisone to the embryo by a more direct route than the maternal circulation cortisone was injected directly into the uterus.

Females were operated on day 13 and in some cases on day 12 as in the preceding section. At first 1.25 mg of cortisone were injected into the uterus between two embryos avoiding puncture of the amniotic sacs. Later the dose was increased to 2.5 mg (the quantity of fluid being 0.10 cc). The results from injections of cortisone, as well as other fluids, are summarized in table 8.

Table 8

Day	Treatment	No. ♀♀ operated	No. of litters aborted	No. of litters exam'd.	No. cl. pal. treated horns	No. cl. pal. control horns
13	1.25 mg cortisone	3	0	3	0/9	0/4
13	2.5 mg cortisone	18	10	8	6/25	2/10*
12	2.5 mg cortisone	4	1	3	0/9	0/7
13	cortisone vehicle	8	1	7	4/20	0/12
12	cortisone vehicle	1	0	1	0/5	0/1
13	physiol. saline plus phenol	1	0	1	0/2	0/3
12	physiol. saline plus phenol	1	0	1	0/4	0/3
13	Physiol. saline alone	9	1	8	3/32	0/15
13	needle only	5	1	4	0/15	0/9

* These two cleft palates occurred in a litter where there were 5 embryos in each horn and it is possible that the horn recorded as control was treated.

With 2.5 mg of cortisone injected on day 13, 6/25 cleft palates were obtained from treated horns and 2/10 in the control horns (this control result, as noted at the foot of table 8, may well have been a mistake -- this is substantiated by the fact that cleft palates have not been recorded for the control horns in any of the other treatments).

Surprisingly, it was found that the injection of 0.10 cc cortisone vehicle (without cortisone) caused 4/20 cleft palates and also that physiological saline caused 3/32 cleft palates. Therefore the injection of 0.10 cc of fluid into the uterus between embryos can cause cleft palate in neighboring embryos, and even in embryos which although in the same horn are not near the injection site.

A series of operations are now being conducted in which a needle is inserted into the uterus in the same manner as for injection but no fluid is injected. So far (bottom of table 8) there are 0/15 cleft palates for treated horns and

0/9 for the control. Should this last series of experiments continue to give negative results, it can be concluded that it is not the operation or needle puncture per se that causes the clefts, and the effect of fluids of varying osmotic pressures will be studied. (Physiological saline is probably hypotonic to amniotic fluid.)

c) Folic acid antagonist. Amino-An-Fol, one of the weaker folic acid antagonists, will cause cleft palate and extensive skeletal abnormalities when injected into pregnant mothers. Preliminary work with hybrid animals has shown that a single dose of one or two mg appears to be fairly effective in causing cleft palate when given on one of the following gestation days: 8, 9, 10, 11, or 12. Superficial examination indicates that individuals with cleft palates caused by Amino-An-Fol may have relatively broad heads. Comparisons between treated and untreated heads are going to be done in order to verify this impression.

6. Publications

During the year two papers arising out of the work of this project have been accepted for publication:

- a) Trasler, D.G., Clark, K.H., and Fraser, F.C. "Absence of cleft palates in the offspring of pregnant mice treated with cortisone after embryonic palate closure". J. Hered. (in press).
- b) Walker, B.E., and Fraser, F.C. "The closure of the secondary palate in mice." J. Embryol. Exp. Morph. (in press).

In addition, the grantee has presented papers based partly on the above work at the Combined Paediatric Meetings (Society for Pediatric Research) Quebec City, June 1955, and at a Conference on Teratology, Cincinnati, January 1956.

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- Walker, B.E. and F.C. Fraser. The closure of the secondary palate in mice. *J. Embryol. Exp. Morph.* (in press).

non-soluble fraction by paper chromatography: alanine, arginine, aspartic acid, glutamic acid, glycine, hydroxyproline, isoleucine, leucine, lysine, methionine, valine, phenylalanine, proline, serine, threonine, tryptophan and tyrosine. Preliminary quantitative data, obtained by quantitative chromatographic techniques, is contained in this report.

3. The main hexose found in the insoluble fraction of enamel is galactose. Smaller quantities of the following monosaccharides were found: glucose, mannose, fucose and glucuronic acid.

APPENDIX H

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: The amino acid and carbohydrate composition of enamel using paper chromatography.

Grantee: Dr. G. Nikiforuk,
University of Toronto.

Project No.: DR-57

Amount of Grant: \$3,358.

SUMMARY OF WORK

The amino acid composition and the carbohydrate components of enamel are being investigated using paper chromatography. A summary of the work follows:

1. Enamel powder (99.9 per cent) was demineralized by dialysis against ethylene diamine tetracetate, pH 7.1, and separated into soluble and non-soluble components. The non-soluble component was hydrolyzed with 6N HCl for the amino acid determination; and with a cation exchange resin for identification of carbohydrate components.

2. The following amino acids have been identified in the non-soluble fraction by paper chromatography: alanine, arginine, aspartic acid, glutamic acid, glycine, hydroxyproline, isoleucine, leucine, lysine, methionine, valine, phenylalanine, proline, serine, threonine, tryptophane and tyrosine. Preliminary quantitative data, obtained by quantitative chromatographic techniques, is contained in this report.

3. The main hexose found in the insoluble fraction of enamel is galactose. Smaller quantities of the following monosaccharides were found: glucose, mannose, fucose and glucuronic acid.

PROGRESS REPORT

Enamel contains about 0.2 to 0.5 per cent organic matter (Stack, 1955), or approximately one-fortieth of that found in dentin. The organic fraction is predominantly protein in nature and also contains carbohydrate components. This report deals with a study of the protein and carbohydrate components of enamel using paper chromatography.

Materials and Methods

1. Preparation and Purification of Powdered Enamel.

Sound extracted teeth were stored in acetone and cleansed by lightly grinding the external surface with a diamond stone to remove all visible stains, pit and fissure material. The cleaned fragments were pulverized in a mill, after which metal particles were removed magnetically. The powder that passed a 100-mesh sieve was found most suitable for purification. Larger particles were more difficult to purify because of a greater dentin content.

Enamel was purified by a flotation technique (Manly and Hodge, 1939). A bromoform-acetone mixture having a density of 2.75 was used to separate enamel (S.G. 2.90) from dentin (S.G. 2.14). Separation was repeated from four to eight times on the enamel fraction until the dentin impurities were reduced to 0.1 - 0.2 per cent "junction" particles. About two-tenths of the area of a "junction" particle consisted of dentin, the remainder of enamel.

Enamel particles were differentiated from dentin on the basis of different refractive indices (dentin 1.56 and enamel 1.60). A liquid with a refractive index of 1.59 prepared by mixing 76 vol.% of α -chloronaphthalene and 18 vol.% of a liquid petroleum was used for this purpose. A mixture of enamel and dentin when placed in this liquid and studied under a microscope reveals a halo called the "Becke line" which appears to enter the enamel particle, but moves out from the dentin particle when the focus is raised.

2. Demineralization.

Pure enamel powder (100 to 300 mgs) was placed in a cellophane dialysis sac. The sac was agitated in a beaker containing 1.5 litres of 0.5 per cent phosphoric acid or

0.25 M ethylene diamine tetracetate, pH 7.1 ± 0.1 and 0.012% zephiran chloride. Demineralization was usually complete in five days and the demineralizing solution was replaced with de-ionized water, changed twice daily for four days. At the end of this period the dialysis membrane is presumed to contain only non-diffusible organic molecules. The insoluble fraction (average yield, 0.22%) is separated from the supernatant by centrifugation for 30 minutes, (R.C.F. = 30,500) washed with alcohol and acetone, dried and stored at -20°C . The supernatant was concentrated to 0.05 ml by lyophilization and used for determination of soluble protein or peptide fractions.

3. Hydrolysis.

Hydrolysis of the insoluble fraction was carried out in a sealed ampule using 5 ml 6N HCl for 48 hours at 110°C . For sugar determination the insoluble organic fraction of enamel was hydrolyzed with styrene type, sulfonic acid cation exchange resin (Dowex 50 - X12) at 80°C for 96 hours (Glegg and Eiding, 1954). These experimental conditions produced maximum hydrolysis of chondroitin sulfate but no destruction of standard sugar solutions. The soluble carbohydrate was separated from the resin by centrifugation and washing. Hexosamines were absorbed on the resin and could be eluted with 0.5N HCl.

4. Chromatography Technique.

Last year's report referred to the marked improvement in the chromatography of amino acids using washed filter paper. The filter paper (Whatman No. 3, $18\frac{3}{4}" \times 22\frac{1}{4}"$) was washed for 52 days according to the method of Connell, Dixon and Hanes (1955). The solvents used for amino acid resolution were (1) n-isopropyl alcohol and water (80-20V/V) and (2) phenol (saturated with an aqueous solution containing 6.3 per cent sodium citrate and 3.7 per cent sodium dihydrogen phosphate) containing 5 per cent (V/V) ethyl acetate. The starting area was exposed for about 30 seconds to vapours of a 25% solution of ammonia. Descending two-dimensional chromatography was used with a period of 4 hours equilibration before addition of solvent to trough. The chromatograms were developed using a detection spray containing 50 mgs ninhydrin dissolved in 75 ml absolute ethanol plus 25 ml 2N acetic acid. For quantitative work the spots from the papergram were carefully excised, the paper flooded

with borate buffer, pH 11.5, the colored spot eluted and developed in a quantitative ninhydrin-hydrindantin reagent according to the method of Connell et al, 1955. A representative area was excised and treated as above for "blank" determinations.

The sugar papergrams were developed in a n-butanol-pyridine-water (3:1:1) solvent. Sugar spots were revealed by dipping the dried paper in an aniline hydrogen oxalate-acetone bath (aniline 0.4% V/V; 1 M oxalic acid 2% V/V; and acetone 97.6% V/V/, followed by heating in an oven at 85°C for 30 minutes.

Findings

1. Insoluble enamel fraction.

Figure I is a papergram of the hydrolysate obtained from 1.00 gram of enamel which yielded 0.1 mg of insoluble fraction of enamel. A chromatogram of known amino acids (0.04 μ M) run under identical conditions is shown in Figure II.

The spots from the papergram containing the experimental and standard amino acids were eluted and the amount of the amino acid(s) determined. Table 1 indicates the amino acids identified and the concentration of the amino acid found in the insoluble organic fraction of enamel in per cent by weight. These results are preliminary and are being repeated.

2. Soluble enamel fraction.

The soluble (in EDTA at pH 7.1) fraction was reduced to a volume of 0.05 ml. A two-dimensional chromatogram of this fraction and the control (diffusate from dialysis membrane without any enamel) was run. Spots in the control chromatogram confirm last year's finding of contaminants resulting from the cellophane membrane. Contamination in the control was too great to permit positive identification of a soluble protein or peptide fraction in enamel. Experiments are in progress to answer this important point.

3. Carbohydrate components of enamel.

Studies so far have been limited to the insoluble fraction of enamel. Figure III indicates that galactose, glucose, mannose, fucose and glucuronic acid are present in

enamel. The dominant hexose in enamel is galactose, of which 50-100 $\mu\text{g/gm}$ was found. The other sugars noted above were found in smaller quantities. For comparative purposes the carbohydrate components in dentin, determined under similar conditions, are noted in Figure IV. This papergram confirms the finding of Leblond (1954) that dentin contains glucuronic acid, galactose and small amounts of other hexoses.

- - - - -

The assistance of Professor C.S. Hanes and Dr. G.E. Connell is gratefully acknowledged. The work on the carbohydrate composition of enamel was done in collaboration with Mr. Ralph Burgess.

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Manly, R.S. and Hodge, H.C. Density and refractive index studies of dental hard tissues. J. Dent. Res. 18: 133, 1939.

Stack, M.V. Organic constituents of enamel. J. Amer. Dent. Assoc. 48: 297, 1955.

Table 1

Quantitative analysis of hydrolysate of purified enamel protein. Amino acids separated on a two-dimensional papergram using N-isopropyl alcohol and buffered phenol as solvents.

Amino acids	Per cent of insoluble fraction	Per cent of total amino acids
L-alanine	2.89	5.1
L-arginine	11.56	20.5
L-aspartic acid	4.5	8.0
(L-cysteine) *		
(L-cystine) *		
L-glutamic acid	4.9	8.6
glycine	4.0	7.2
L-histidine	?	
L-hydroxyproline	2.6	4.0
(L-isoleucine) **		
(L-leucine) **	6.6	11.6
L-lysine	6.9	12.3
(L-methionine) **		
(L-valine) **	1.5	3.1
L-phenylalanine	0.7	1.2
L-proline	3.0	5.6
L-serine	2.3	4.0
L-threonine	1.3	2.4
L-tryptophane	+	
L-tyrosine	3.2	5.7

Total weight of amino acids, 56.35 μ g.

Total weight of insoluble organic fraction, 100 μ g.

- * Cystine was not recovered in this experiment, probably due to oxidation.
- ** Leucine, isoleucine and valine, methionine were not separated on the "papergram". In calculating the concentration it was assumed the amino acids occurred in equal proportions.

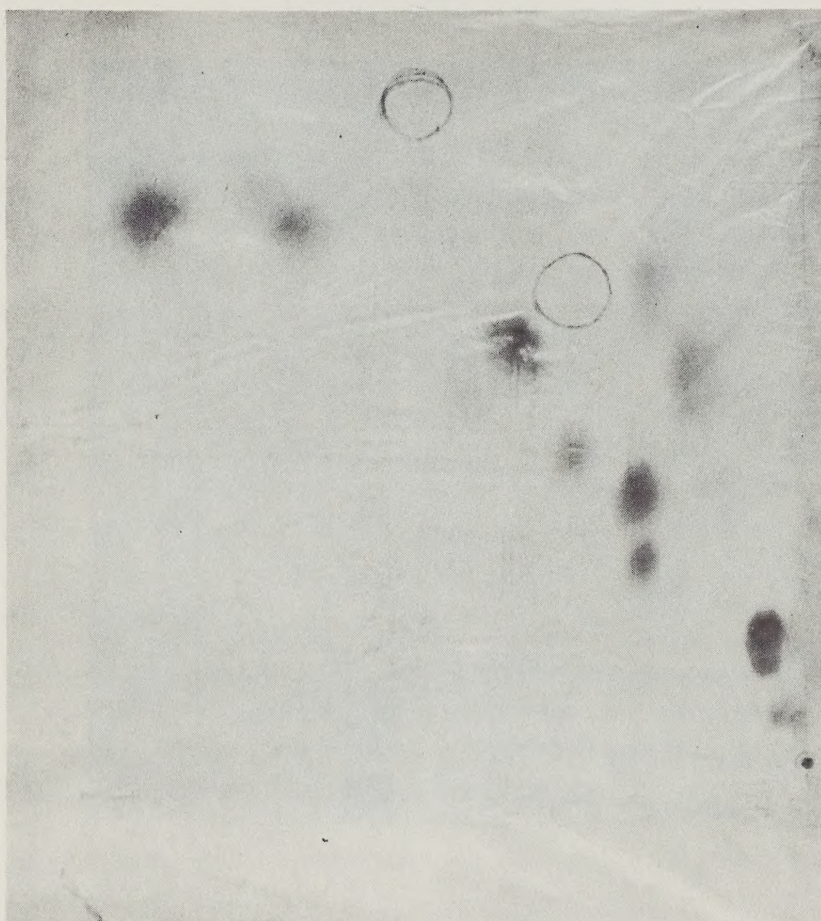


FIGURE 1. 'PAPERGRAM' OF THE PROTEIN HYDROLYSATE FROM 1 GRAM OF ENAMEL WHICH YIELDED 0.1 MG OF INSOLUBLE ORGANIC MATERIAL. HISTIDINE AND CYSTINE WERE NOT IDENTIFIED ON THIS CHROMATOGRAM.

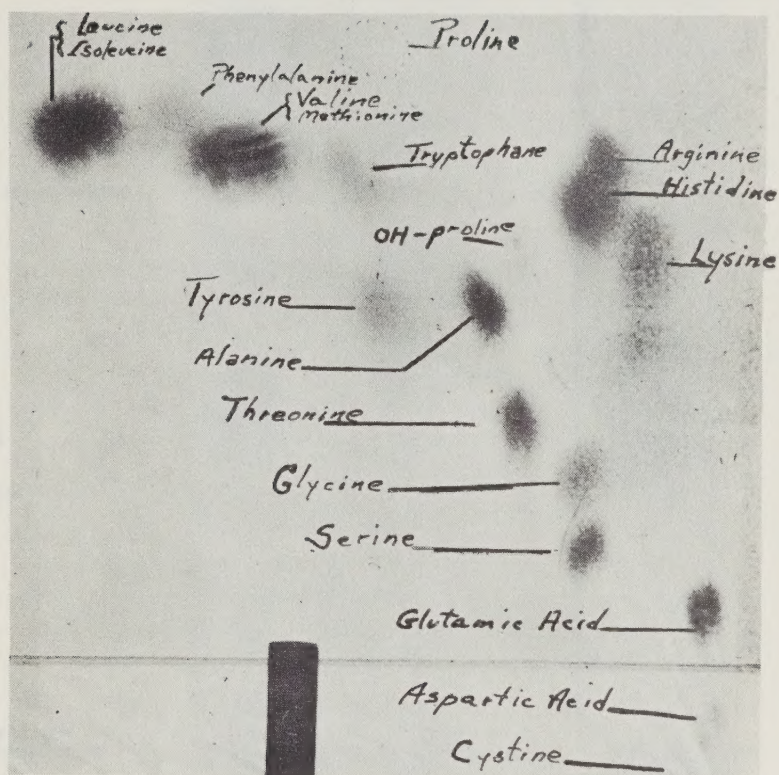


FIGURE 11. A TWO-DIMENSIONAL CHROMATOGRAPHIC PATTERN OF TWENTY AMINO ACIDS ($0.04 \mu\text{M}$) USING PROPANOL AND PHENOL AS THE TWO SOLVENTS. LEUCINE, ISOLEUCINE AND VALINE, METHIONINE ARE NOT SEPARATED BY THIS SYSTEM.

LACTONE OF
GLUCURONIC ACID

RIBOSE

FUCOSE

N-ACETYL-GLUCOSAMINE

MANNOSE
FRUCTOSE

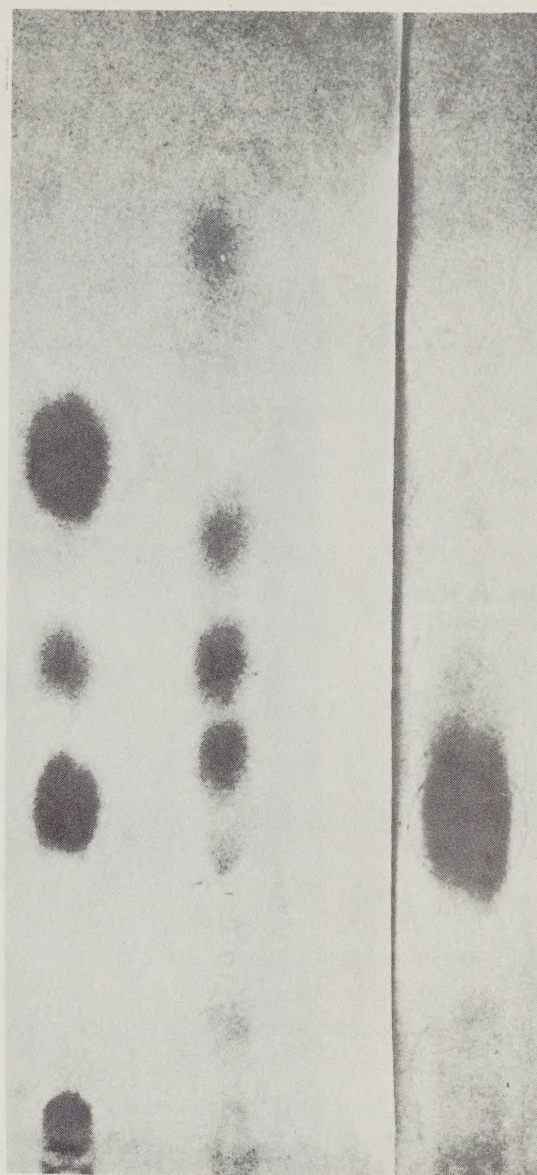
GLUCOSE

GALACTOSE

GLUCOSAMINE

GLUCURONIC AND
GALACTURONIC ACID

STANDARD SOLUTIONS
(100% OF EACH SUGAR)



↑
HYDROLYSATE FROM 4 GRAMS OF
ENAMEL POWDER (0.109% INSOL-
UBLE ORGANIC MATTER)

N-BUTANOL - PYRIDINE - WATER
(3:1:1)

FIGURE III. 'PAPERGRAM' OF HYDROLYSATE OF THE INSOLUBLE FRACTION OF ENAMEL FOR DETERMINATION OF MONO-SACCHARIDES PRESENT. THE MARKERS ARE NOTED ON THE LEFT SIDE OF THE EXPERIMENTAL COLUMN.

LACTONE OF
GLUCURONIC ACID

FUCOSE

MANNOSE

GLUCOSE

GLUCOSAMINE

GLUCURONIC ACID

STANDARD SOLUTIONS
(50% OF EACH SUGAR)

RIBOSE

N-ACETYL-GLUCOSAMINE

FRUCTOSE

GALACTOSE

GALACTURONIC ACID

STANDARD SOLUTIONS
(50% OF EACH SUGAR)

HYDROLYSATE
FROM 1 GRAM
OF DENTIN

N-BUTANOL - PYRIDINE - WATER
(3:1:1)

FIGURE IV. CHROMATOGRAPHIC PATTERN OF MONOSACCHARIDES PRESENT IN THE ORGANIC COMPONENT OF DENTIN. MARKERS ARE ON THE LEFT AND RIGHT SIDE OF THE EXPERIMENTAL COLUMN.

APPENDIX I

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Secretion of protein in saliva.

Grantee: Dr. A. S. V. Burgen,
McGill University.

Project No.: DR-61 Amount of Grant: \$3,400.

SUMMARY OF WORK

A method for continuously recording protein concentrations in saliva by ultra violet absorption has been developed. The protein secretion curves obtained show four phases. (1) An initial rapid peak due to after secretion from the previous experiment; (2) a low concentration trough passing into (3) a slowly increasing secondary rise of protein secretion (4) a slow fall off in protein secretion as the internal concentration in the gland is depleted.

The rise in concentration in the secondary peak develops slowly with a half time of 30-60 seconds. A similar time of development occurs on changing from a low to a high rate of secretion. On changing down in rate of secretion the protein concentration falls to a lower level with a similar time course. This time is independent of the absolute rate of secretion or stimulation. Further analysis of this rise and fall time is proceeding and is connected to throw light on the mechanism of secretion and rises steeply with rate of flow so that exhaustion of the protein stores has an approximate time course of 0.3 min^{-1} at a flow rate of 0.5 ml/g/min , which falls off to $\sim 0.013 \text{ min}^{-1}$ at 0.05 ml/g/min . Methods of measuring the total gland protein store are being investigated. Work is progressing steadily along these lines.

In addition a beginning has been made on a study of the protein components in saliva by paper electrophoresis. So far the results have not been satisfactory owing to the presence of a high proportion of immobile protein (mucoprotein). A number of lines are being tried to overcome these difficulties.

PROGRESS REPORT

The first part of the project was to develop a satisfactory method for continuously recording protein concentrations in saliva. This has been successfully completed. We have built a recording logarithmic ultra-violet photometer whose output is substantially linear with protein concentration. The absorption is measured in the 260-290 millimicron band. The light source is a hot cathode hydrogen lamp and the band selected by a combination of filters (N1 SO₄, Dimethyl diazaheptene, Corning 984). The quartz windowed flow cuvette is replaceable and has been made in thicknesses of 1-4 mm. The standard cell is 1.5 mm. and has a range of 0-2.5% protein and a capacity of 35 μ l. At moderate rates of parotid secretion in the dog the recording lag is 2-5 seconds. Examples of the type of record obtained when this cell is connected to a cannula in the dog's parotid duct are appended. These photographs show the characteristic features of the initiation of protein secretion. (1) A rapid ejection of a moderate concentration of protein (2) followed by an equally rapid decline in concentration (3) a slow secondary build-up to a more or less steady level (4) a slow decline in protein concentration. The initial peak is correlated with the rate of stimulation used and hence the concentration of protein reached in the previous run. From a number of points of evidence it seems likely that the first peak is due to protein secreted in the lumen of the acini after secretion of water has ceased. This material is then passively washed out with the newly secreted saliva.

The rise in concentration in the secondary peak develops slowly with a half time of 30-60 seconds. A similar time of development occurs on changing from a low to a high rate of secretion. On changing down in rate of secretion the protein concentration falls to a lower level with a similar time course. This time is independent of the absolute rate of secretion or stimulation. Further analysis of this rise and fall time is proceeding and is expected to throw light on the mechanism of secretion and rises steeply with rate of flow so that exhaustion of the protein stores has an approximate time course of 0.3 min^{-1} at a flow rate of 0.5 ml/g/min. which falls off to $\sim 0.013 \text{ min}^{-1}$ at 0.05 ml/g/min. Methods of measuring the total gland protein store are being investigated. Work is progressing steadily along these lines.

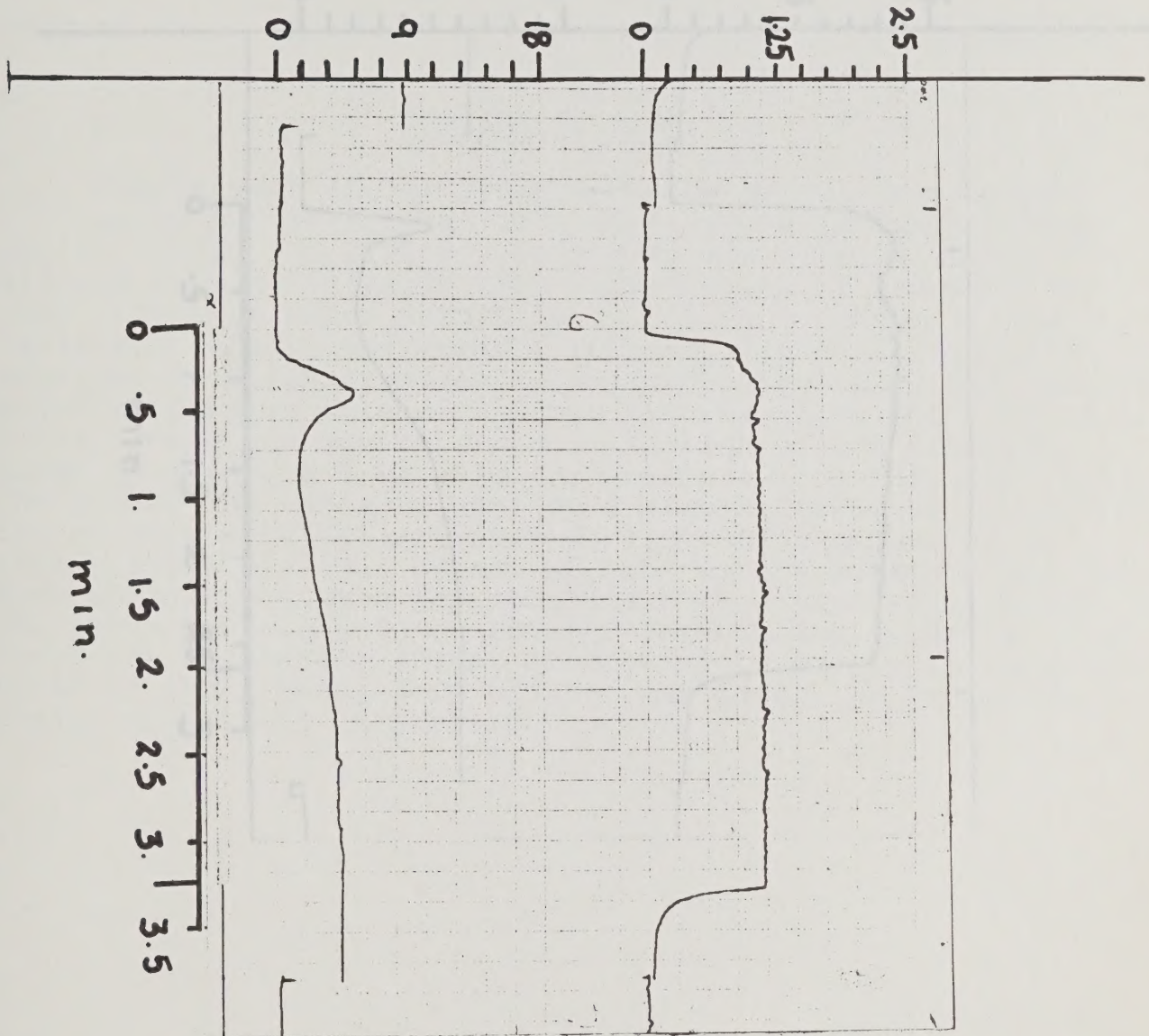
In addition a beginning has been made on a study of the protein components in saliva by paper electrophoresis. So far the results have not been satisfactory owing to the presence of a high proportion of immobile protein (mucoprotein). A number of lines are being tried that may overcome these difficulties.

Protein
Concentration

Rate of Saliva
Secretion

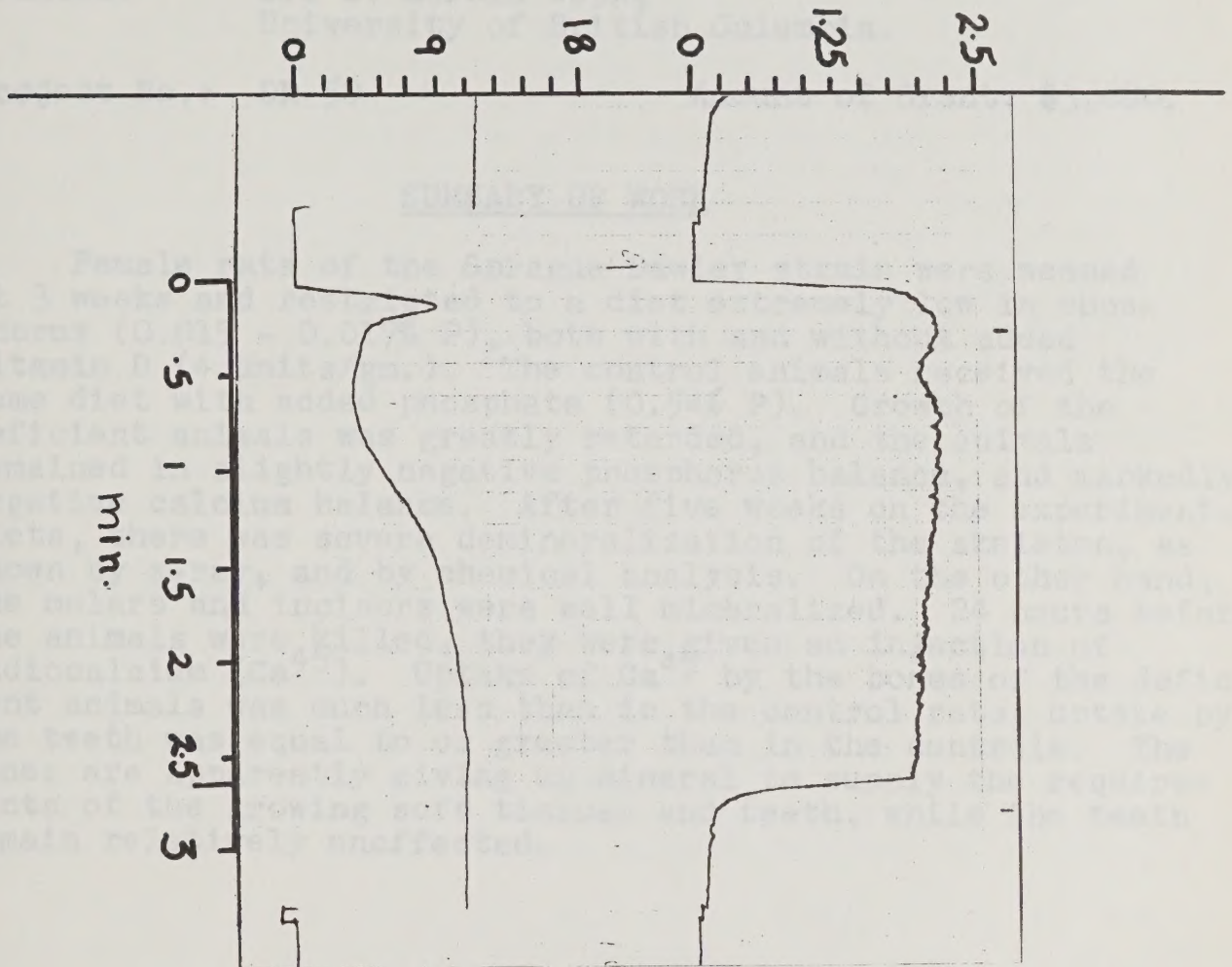
gms/L.

ml./min.



Protein
Concentration
gms/L

Rate of Saliva
Secretion
ml./min



APPENDIX J

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Effect of phosphorus deficiency on bones and teeth.

Grantee: Dr. D. Harold Copp,
University of British Columbia.

Project No.: DR-59 Amount of Grant: \$3,680.

SUMMARY OF WORK

Female rats of the Sprague-Dawley strain were weaned at 3 weeks and restricted to a diet extremely low in phosphorus (0.015 - 0.017% P), both with and without added vitamin D (4 units/gm.). The control animals received the same diet with added phosphate (0.54% P). Growth of the deficient animals was greatly retarded, and the animals remained in slightly negative phosphorus balance, and markedly negative calcium balance. After five weeks on the experimental diets, there was severe demineralization of the skeleton, as shown by x-ray, and by chemical analysis. On the other hand, the molars and incisors were well mineralized. 24 hours before the animals were killed, they were given an injection of radiocalcium (Ca^{45}). Uptake of Ca^{45} by the bones of the deficient animals was much less than in the control rats; uptake by the teeth was equal to or greater than in the controls. The bones are apparently giving up mineral to supply the requirements of the growing soft tissues and teeth, while the teeth remain relatively unaffected.

PROGRESS REPORT

Introduction.

Day and McCollum (1) observed that rats restricted to a diet very low in phosphorus developed severe signs of deficiency within a few weeks, with loss of mineral and severe rarefaction of the skeleton. Death occurred after 6-8 weeks from collapse of the softened rib cage. The histological changes resembled those observed in advanced rickets (2-4), although the deficiency developed even in the presence of adequate amounts of Vitamin D. Although the formation and calcification of dentine is somewhat retarded in the deficient animals (3), gross observations indicated that there was no apparent decrease in the density of the mineral present in teeth. Studies with Sr^{90} (5) and Ca^{45} (6) have indicated changes in the uptake and retention of these isotopes in the deficient animals.

In the following experiments, these effects have been studied in more detail, and the effect of presence or absence of vitamin D has been investigated.

Experimental Procedure.

Female rats of the Sprague-Dawley strain were weaned at 3 weeks, and were then restricted to one of the experimental diets described in Table 1 for a period of 5 weeks. Food consumption was measured, and the output of calcium and phosphorus in urine and feces was measured, as well as the growth in body weight. Growth curves are shown in Figure 1, phosphorus balance in Figure 2, and calcium balance in Figure 3.

After 34 days, each rat received a dose of 0.5 ml. isotopic saline containing 5 microcuries of Ca^{45} and 0.3 mg. calcium. This was injected intraperitoneally. They were killed 24 hours later (i.e. 5 weeks after the beginning of the experimental feeding and 8 weeks after birth). Blood samples were taken at the time of autopsy and analyzed for calcium by the method of EDTA titration (7). A representative animal from each group was skinned and eviscerated, and x-ray photographs were taken with all animals exposed together on the same plate so that the exposures would be identical. These x-ray photographs are shown in Figures 4 - 7.

Each animal was autopsied, and the following tissues were taken for analysis of calcium, phosphorus and Ca^{45} : femur, scapula, molars, incisors, liver, muscle (an aliquot was taken from the thigh), skin, and brain. The urine and feces were also analyzed for Ca^{45} . The phosphorus present in various

organs is shown in Table 2; the phosphorus concentration in tissues is shown in Table 3, and the calcium content of the representative bones and teeth is shown in Table 4. Table 5 gives the per cent of the dose of Ca^{45} present in various bones and teeth and excreta 24 hours after administration.

Results.

Growth and mineral balance (see Figures 1-3)

Rats restricted to the phosphorus deficient diet show a marked retardation of growth, slight negative phosphorus balance, and quite marked negative calcium balance. The rats fed the phosphorus supplemented control diet grew rapidly and normally, and were in marked positive calcium and phosphorus balance. The presence or absence of vitamin D did not appear to have significant effect on either.

Phosphorus content of tissues (see Tables 2 and 3)

The acid soluble phosphate of serum is reduced in the deficient animals, but otherwise, there does not appear to be any significant reduction of the phosphorus content of the soft tissues. This is not surprising in view of the very essential function of phosphorus in living cells. Since the animals are in negative phosphorus balance, this element must have been lost from the skeleton, and this is evident from the values for femur and scapula. The teeth are less affected.

Calcium content of the bones and teeth (See Table 4)

The bones of the deficient animals were quite soft and pliable, and appeared to contain very little bone mineral. This was confirmed by the chemical analyses of femur and scapula, which show a striking reduction in calcium content. The extreme demineralization was also evident in the x-ray photographs. On the other hand, both molars and incisors were well mineralized and appeared to be quite hard. The calcium content was also comparable to that of the control rats when allowance was made for the difference in size. The teeth evidently do not contribute mineral for maintenance of phosphorus in the soft tissues of the deficient rats, as do the bones. It is of interest that the incisors, which continue to grow during the experimental period, also continue to deposit fresh mineral - both in enamel and dentine.

Uptake and retention of radiocalcium (Ca^{45}) (see Table 5)

Twenty four hours after the administration of Ca^{45} by intraperitoneal injection, over 20% of the dose had been excreted in the urine of the phosphorus deficient rats as compared to 0.25% in the urine of the controls. The Ca^{45} retained in femur and scapula of the deficient animals was less than half that in the controls, while the uptake in molars and incisors was at least as great. The phosphorus deficiency apparently reduces the ability of the bones to retain calcium, but does not affect the teeth.

X-ray photographs (see Figures 4-7)

The most striking feature of the x-ray photographs of the skeleton of the deficient rat is the extreme demineralization of the skeleton, and particularly of the vertebral column and ribs, when compared to the 3 week old animal at the onset of the experiment, and the 8 week old control rat. Examination of the skull of the deficient animal indicates that the teeth - both molars and incisors - have good density, while the alveolar sockets are almost completely demineralized.

Conclusions

When young growing rats are restricted to a diet which is extremely low in phosphorus, growth is retarded, and there is a progressive loss of phosphorus and calcium from the body. The phosphorus of the soft tissues is apparently maintained at the expense of the skeleton, which becomes extremely demineralized. On the other hand, the teeth do not appear to contribute mineral to soft tissues, but remain well mineralized, and indeed the growing incisor continues to deposit new mineral. These effects have been demonstrated by chemical analyses and by x-ray photographs. In the deficient rats, retention of Ca^{45} by femur and scapula at 24 hours is reduced, but retention of Ca^{45} by molars and incisors is at least as great as in the controls. It would appear that the teeth are relatively immune from the mineral mobilizing forces which are operating to maintain phosphorus homeostasis in these deficient animals, and this raises some interesting questions concerning the mechanisms involved. Absence of vitamin D did not appear to affect significantly either deficient or control animals. In the rat, it is difficult to produce low vitamin D rickets when the calcium and phosphorus intake is optimal; in severe phosphorus deficiency the effects of mineral deprivation apparently are more important than the lack of the vitamin.

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TABLE 1.Composition of the Experimental Diets.1. Phosphorus Deficient Diet with Vitamin D. (0.016% P)

Sucrose	60%
Purified Beef Fibrin	20%
Vitamin A Palmitate 1,000 I.U./ gram	1%
Vitamin D 400 I.U./gram	1%
Potassium Chloride	1.5%
Magnesium Sulfate $.7H_2O$	.3%
Calcium Carbonate	1.0%
Sodium Chloride	1.0%
* Vitamin Mix.	1.0%
** Trace Element Mix.	1.0%
Hydrogenated Cottonseed Oil (Crisco)	10%

2. Deficient Diet with no vitamin D.

Same as above except Vitamin D. was omitted.

3. Control Phosphorus Supplemented Diet with Vitamin D. (0.54% P)

Sucrose	60%
Purified Beef Fibrin	20%
Hydrogenated Cottonseed Oil (Crisco)	10%
Vitamin A Palmitate 10,000 I.U./gm.	1%
Vitamin D 400 I.U./gm.	1%
Potassium Chloride	1.5%
Magnesium Sulfate $.7H_2O$	.3%
Calcium Carbonate	1.0%
Sodium Phosphate	2.4%
Vitamin Mixture	1.0%
Trace Element Mixture	1.0%

4. Control Phosphorus Supplemented Diet with no Vitamin D.

Same as Control except Vitamin D was omitted

- * Vitamin Mix as in Coleman et al. (4)
- ** Trace Element Mix.

TABLE 2.

Phosphorus Content of Organs of Onset, Phosphorus Deficient and Control Rats (mgms) *

	At Onset of Expt.	Phosphorus Deficient Rats.		Control Phosphorus Supplemented Rats.	
	3 weeks old	With Vit. D.	No Vit. D.	With Vit. D.	No Vit. D.
Number of rats in group	10	10	5	10	10
<u>SOFT TISSUES:</u>					
Muscle	16.1 ± 1.2	30.9 ± 1.3	22.4 ± 1.8	81.6 ± 5.8	51.3 ± 1.2
Liver	5.71 ± 0.31	4.68 ± 0.04	3.67 ± 0.07	9.28 ± 0.27	10.36 ± 0.08
Skin	3.12 ± 0.41	2.16 ± 0.13	-	4.11 ± 0.11	4.16 ± 0.06
Brain	3.42	2.21 ± 0.38		2.51 ± 0.25	2.20 ± 0.49
<u>BONES</u>					
Femur	3.99 ± 0.19	2.60 ± 0.24	3.80 ± 0.12	20.4 ± 0.20	20.90 ± 2.50
Scapula	1.31 ± 0.66	1.20 ± 0.11	0.97 ± 0.08	5.3 ± 0.50	5.4 ± 0.22
<u>TEETH</u>					
Molars	5.15 ± 0.34	4.64 ± 0.02	-	15.1 ± 1.1	14.6 ± 0.60
Incisors	4.34 ± 0.24	4.65 ± 0.003	-	16.8 ± 0.01	17.43 ± 0.63

* Mean values ± standard error.

TABLE 3.

Phosphorus concentration in the tissues of onset, phosphorus deficient and control rats.

	Number of rats in group	At onset of expt. 3 weeks old just weaned	Phosphorus Deficient Rats		Control Rats	
			With vit. D.	No vit. D.	With vit. D.	No vit. D.
		10	10	5	10	10
<u>SOFT TISSUES (mg.%)</u>						
Serum		3.01 ± 0.11	1.27 ± .17	.91 ± .19	3.28 ± 1.9	3.02 ± .35
Muscle		102 ± 7	98 ± 4	82 ± 11	116 ± 16	82 ± 3
Liver		178 ± 49	158 ± 7	165 ± 4	144 ± 3	143 ± 20
Brain		296 ± 40	184 ± 34		184 ± 18	179 ± 47
<u>BONES (gms.%)</u>						
Femur		2.48 ± 0.29	1.16 ± 0.21	1.50 ± 0.13	4.09 ± 0.27	4.07 ± 0.70
Scapula		3.08 ± 0.36	0.75 ± 0.16	0.89 ± 0.71	2.20 ± 0.14	4.33 ± 0.53

* Values given are averages for each group ± standard error.

TABLE 4.

Calcium Content of the Bones and Teeth of Onset, Phosphorus Deficient and Control Rats *

	At onset of expt. 3 weeks old just weaned	Phosphorus Deficient Rats		Control Rats	
		With vit. D	No vit. D	With vit. D	No vit. D
Number of rats in group	10	10	5	10	10
<u>BLOOD SERUM</u> mg. %	9.3 ± 0.29	10.0 ± 0.2	9.5 ± 0.2	9.2 ± 0.3	8.6 ± 0.5
<u>BONES</u>					
Femur	11.17 ± 0.44	5.5 ± 1.6	6.4 ± 1.3	45.3 ± 1.6	49.3 ± 4.7
Scapula	5.08 ± 0.38	2.18 ± 0.29	2.10 ± 0.26	13.5 ± 0.9	11.2 ± 1.2
<u>TEETH</u>					
Molars	19.73 ± 0.70	26.6 ± 1.8	-	35.1 ± 1.1	38.6 ± 1.1
Incisors	15.67 ± 0.53	27.4 ± 1.7	-	44.0 ± 1.4	37.2 ± 0.6
<u>CARCASS</u>	317	235	246	1,265	997

* Values given are averages for each group ± standard error.

TABLE 5.

Per cent of the administered dose of Ca^{45} present 24 hrs. after injection.

	<u>Phosphorus Deficient</u>		<u>Control Diet</u>	
	<u>With vit. D.</u>	<u>No vit. D</u>	<u>With vit. D.</u>	<u>No vit. D.</u>
Number of rats in group	10	5	8	8
<u>BONES</u>				
Femur	1.91 $\pm$ 0.16	1.82 $\pm$ 0.17	4.10 $\pm$ 0.28	3.84 $\pm$ 0.12
Scapula	0.55 $\pm$ 0.05	0.42 $\pm$ 0.03	0.94 $\pm$ 0.09	1.15 $\pm$ 0.06
<u>TEETH</u>				
Molars	0.65 $\pm$ 0.07	0.90 $\pm$ 0.12	0.45 $\pm$ 0.04	0.60 $\pm$ 0.05
Incisors	2.13 $\pm$ 0.19	3.87 $\pm$ 0.51	2.03 $\pm$ 0.11	2.03 $\pm$ 0.11
<u>EXCRETA</u>				
Feces	1.41 $\pm$ 0.31	-	0.84 $\pm$ 0.07	2.64 $\pm$ 0.29
Urine	20.8 $\pm$ 0.6	-	0.21 $\pm$ 0.05	0.25 $\pm$ 0.05

* Values given are averages for each group $\pm$ standard error.

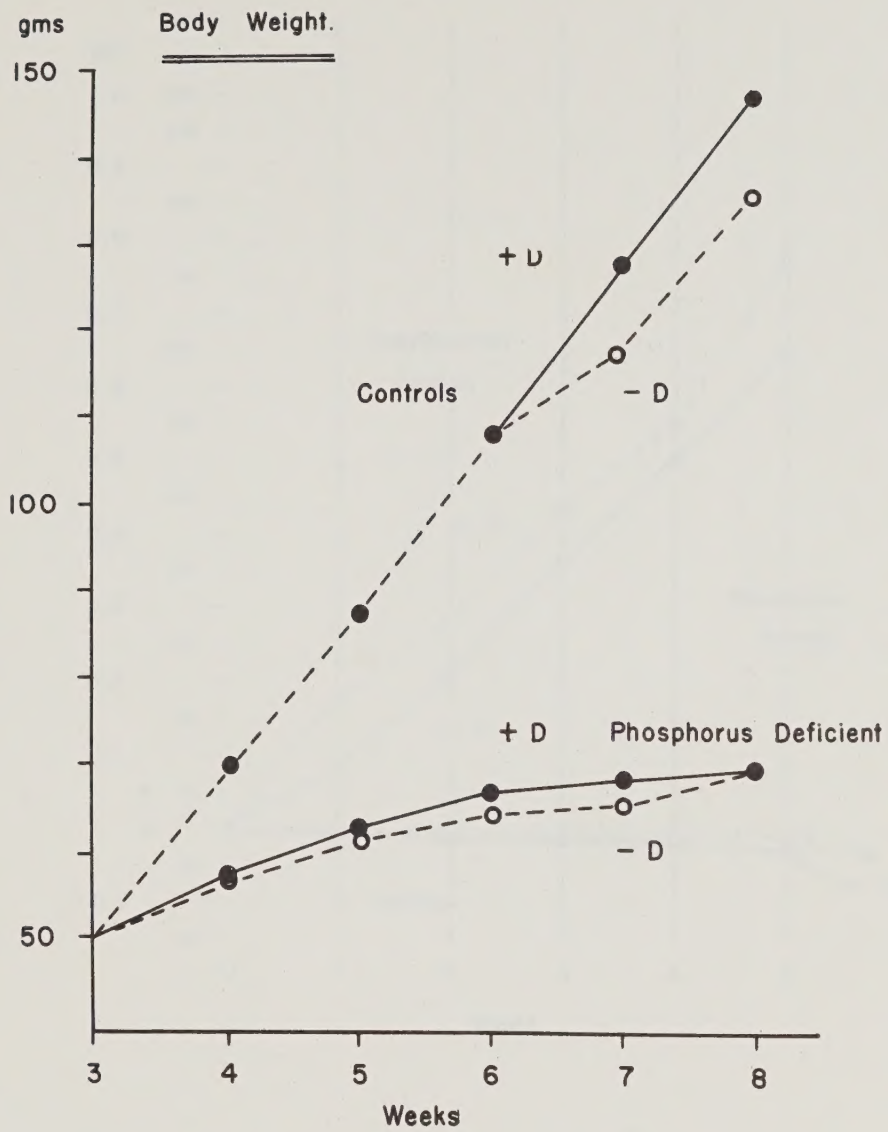


Figure 1. Growth Curves of Phosphorus Deficient and Control Rats.

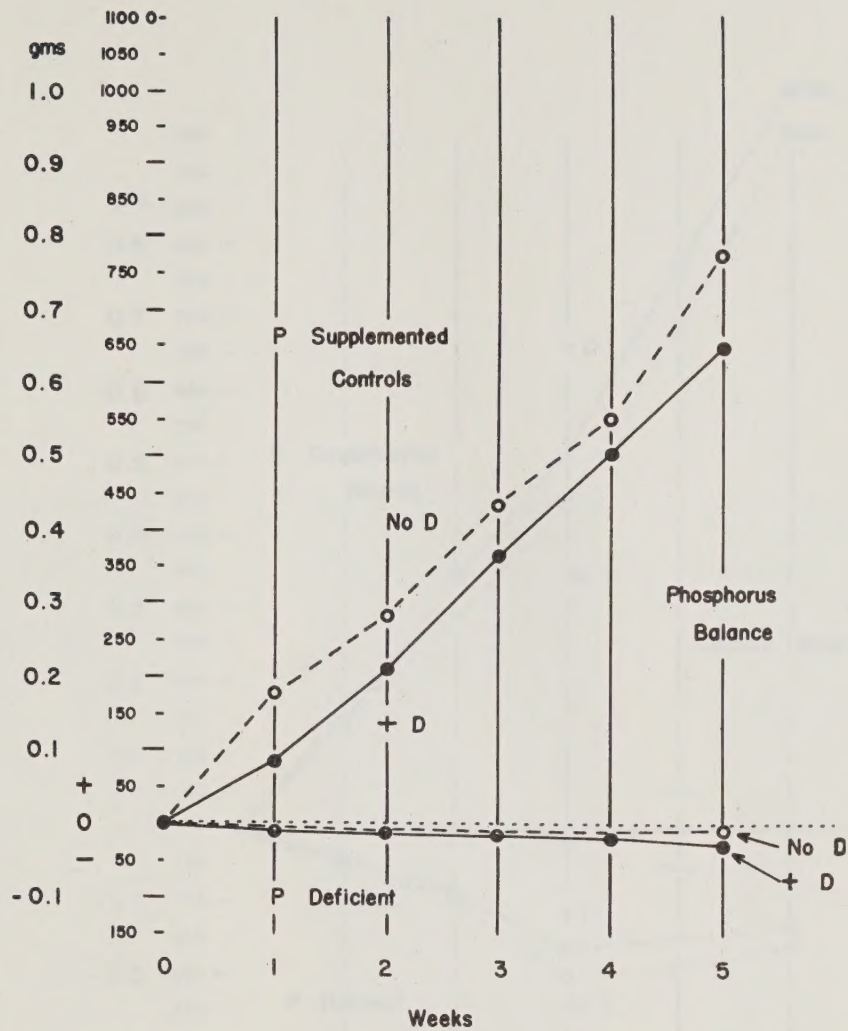


Figure 2. Phosphorus balance of phosphorus deficient and control rats.

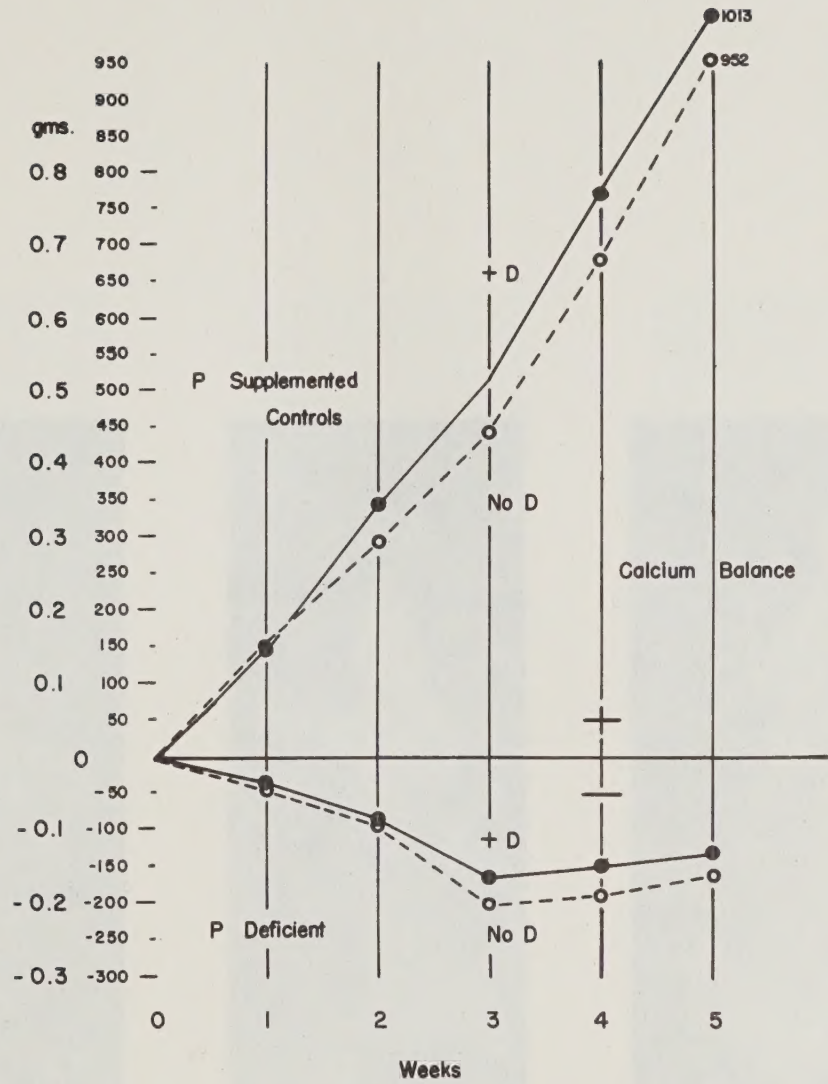


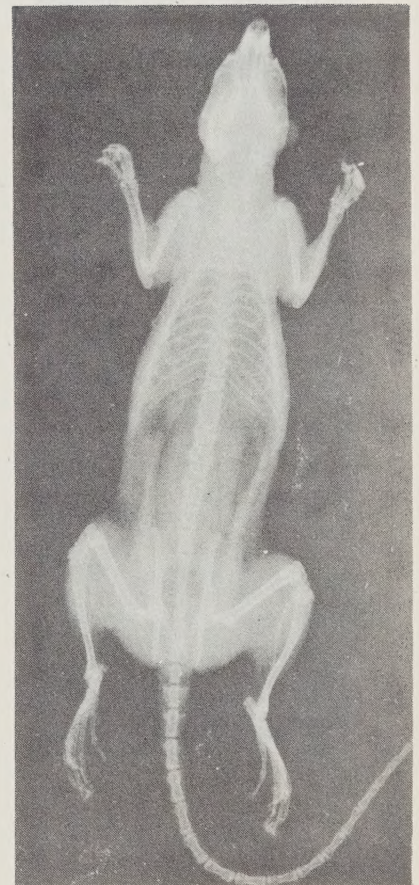
Figure 3. Calcium balance of phosphorus deficient and control rats.



a) AT ONSET OF EXPT.
3 weeks old

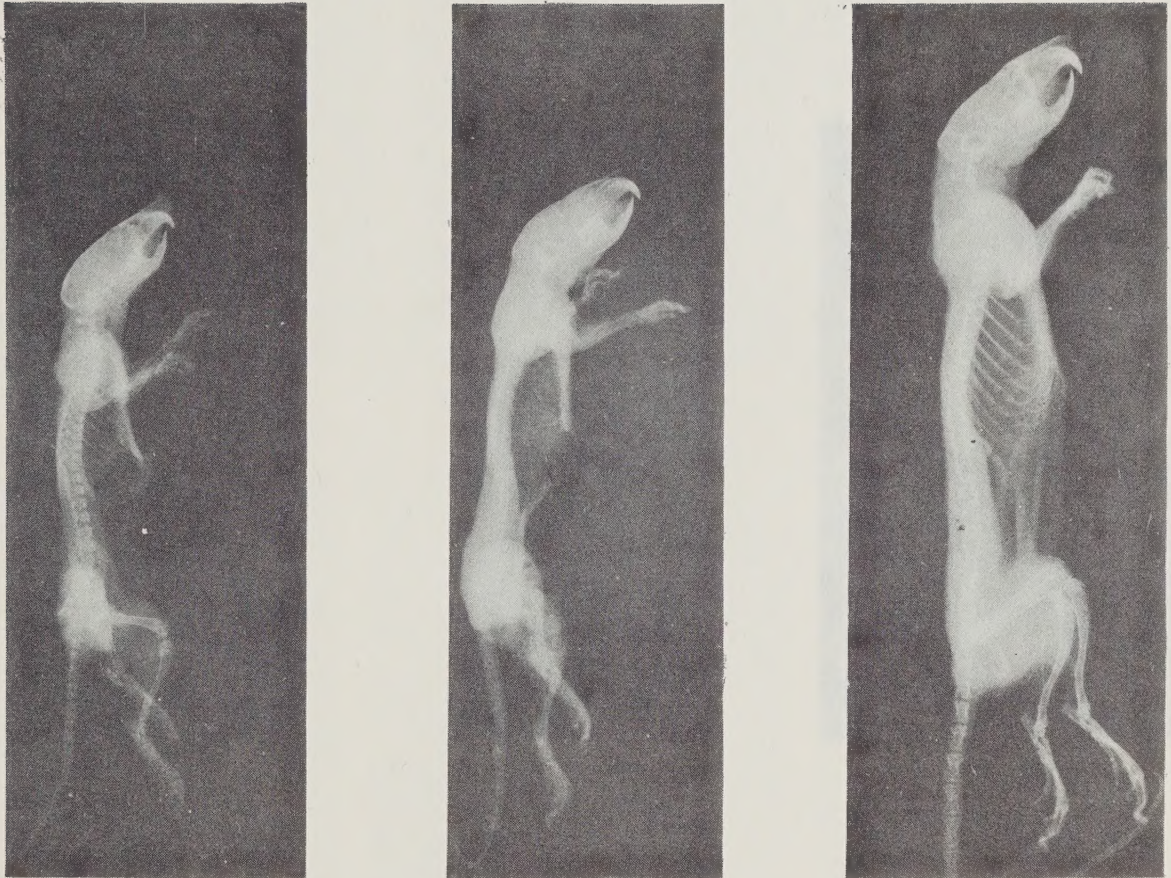


b) PHOSPHORUS DEFICIENT
8 weeks old



c) CONTROL DIET
8 weeks old

Figure 4. Anteroposterior x-ray views of onset, deficient and control rats.



a) AT ONSET OF EXPT.

b) PHOSPHORUS DEFICIENT

c) CONTROL DIET

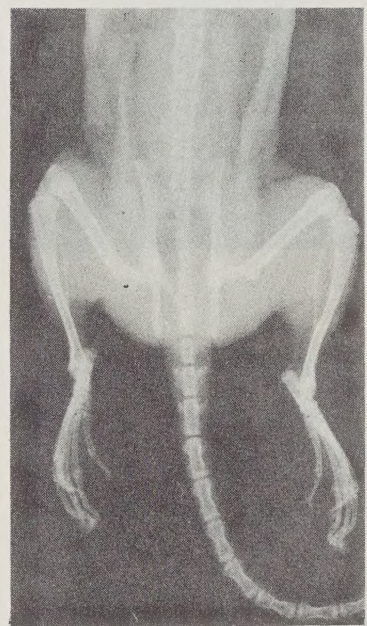
Figure 5. Lateral x-ray views of onset, deficient and control rats.



a) AT ONSET OF EXPT.
3 weeks old

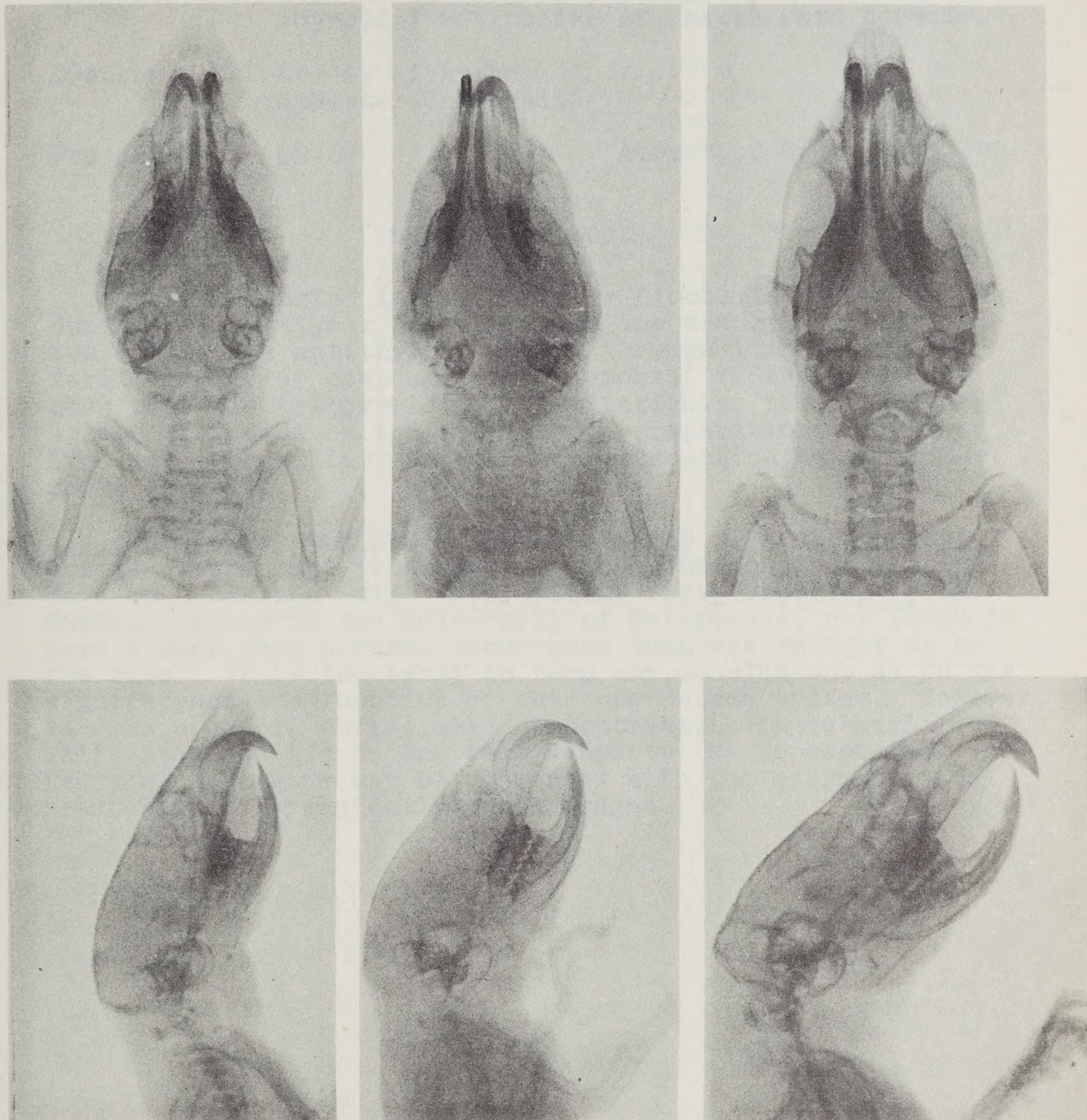


b) PHOSPHORUS DEFICIENT
8 weeks old



c) CONTROL DIET
8 weeks old

Figure 6. x-ray views of the hindquarters of onset, deficient and control rats.



a) AT ONSET OF EXPT.
3 weeks old

b) PHOSPHORUS DEFICIENT
8 weeks old

c) CONTROL DIET
8 weeks old

Figure 7. X-ray views of skulls of onset, deficient and control rats.

APPENDIX K

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Role of the salivary glands in the extra-thyroidal metabolism of the thyroid hormone.

Grantee: Dr. S. Kirkwood
McMaster University

Project No.: DR-62 **Amount of Grant:** \$3,200.

SUMMARY OF WORK

Work conducted in the McMaster Biochemistry Laboratory during the past year has confirmed the theory that iodide ion exerts its antithyroid action through its ability to form a molecular compound with elemental iodine. It has been possible to correlate, quantitatively, the inhibitory action of iodide on isolated enzyme preparations, prepared from rat submaxillary tissue, with its action on the intact mouse thyroid.

Extensive studies on the occurrence of a mitochondrial enzyme system that produces protein-bound moniodotyrosine have been carried out. This system, first reported by the Chaikoff group at the University of California, was found to have a much more general occurrence than was thought to be the case. It is too early to form any conclusions as to the significance and function of this new enzyme system. However, it does occur in appreciable concentrations in submaxillary salivary gland tissue and this supports the concept that salivary gland tissue may be concerned with the extrathyroidal metabolism of organically-bound iodine.

Recent studies in our laboratory have been directed toward the goal of determining the relationship of these two enzyme systems. In addition we have conducted some work with the view of shedding light on a controversy that has arisen in the literature on the subject of the mechanism of the antithyroid action of iodide ion. The latter work will be reported soon, since it seems to have reached a definite conclusion and is quite separate from the work on the two enzyme systems.

The Mechanism of the Antithyroid Action of Iodide Ion

It has been known for some time that the administration of excess iodide ion to both animals and human subjects results in inhibition of thyroxine synthesis. This accumulation is a result of

PROGRESS REPORT

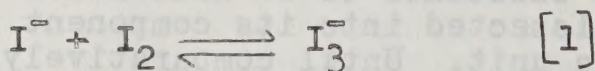
One of the most fruitful methods for studying the chemical processes in the body is the technique of isolating enzyme systems that are responsible for causing the reactions under study. This allows the complete pathway of synthesis of any essential substance to be determined since the whole process can be dissected into its component parts and each part studied as a unit. Until comparatively recently all attempts to apply this method to the study of the process whereby the thyroid gland synthesized its hormone were unsuccessful. However, recently two systems that carry out parts of the synthesis of thyroxine have been isolated from the thyroid gland. The first of these was isolated in the biochemistry laboratory at McMaster University. It was found that ground preparations of the thyroid gland, supplemented with cupric ion and tyrosine, synthesized moniodotyrosine (MIT), the first intermediate in the synthesis of thyroxine in the gland (1). More recently the group working with Chaikoff at the University of California has demonstrated an enzyme system in particles isolated from thyroid tissue that also catalyzes the formation of MIT (2). These so-called "particulate" preparations consist of microscopic particles that occur in the cytoplasm of all cells. These particles, called "mitochondria", can be separated from the rest of the tissue material by the process of differential centrifugation and the enzyme systems resident in them can be studied in detail. It is felt that mitochondria represent nothing more nor less than assemblies of enzyme systems and it is often possible to persuade enzyme systems to act in the mitochondria that will not function if they are brought into solution. The isolation of these two systems from thyroid is of considerable interest since, although they both achieve the synthesis of MIT, the McMaster system synthesizes free MIT while the Chaikoff system synthesizes MIT in protein-bound form. The question then arises as to which system represents the true state of affairs in the thyroid gland.

Recent studies in our laboratory have been directed toward the goal of determining the relationship of these two enzyme systems. In addition we have conducted some work with the view of shedding light on a controversy that has arisen in the literature on the subject of the mechanism of the antithyroid action of iodide ion. The latter work will be described first, since it seems to have reached a definite conclusion and is quite separate from the work on the two enzyme systems.

The Mechanism of the Antithyroid Action of Iodide Ion.

It has been known for some time that the administration of excess iodide ion to both animals and human subjects results in inhibition of thyroxine synthesis. This anomalous behaviour of

iodide ion has been a source of puzzlement among thyroidologists since it is very surprising that a substance that goes into the make-up of the thyroid hormone should also inhibit the process whereby it is synthesized. Some time ago Fawcett and Kirkwood (3) advanced the idea that iodide inhibited the process of thyroxine synthesis by virtue of its ability to combine with elemental iodine thus:



A considerable body of evidence was produced that supported the above concept.

Recently Wollman and Scow (4) have challenged this mechanism for the antithyroid action of iodide ion on the basis that it cannot quantitatively explain the inhibitory action observed when varying concentrations of iodide are administered to intact mice. Since the equilibrium constant for reaction [1] is known, the degree of inhibition produced by any concentration of iodide can be predicted, if it acts through the triiodide mechanism. Wollman and Scow administered a graded series of concentrations of iodide to mice, estimated the concentration of iodide in their thyroids by the tracer technique, and then calculated the expected inhibition and compared it with the measured inhibition. The results showed that while the Fawcett and Kirkwood mechanism (hereafter referred to as the triiodide mechanism) would account fairly well for the degree of inhibition produced by large concentrations of iodide ion, it would not even approximate the degree of inhibition produced by concentrations of iodide that just produced observable inhibition of organic binding in the thyroid gland. They therefore came to the conclusion that was as yet no satisfactory explanation for the inhibitory action of iodide ion on the thyroid gland.

In order to resolve this question we have made use of the soluble enzyme system previously described. If this system does represent the first and second steps of thyroxine synthesis, then the locus of action of iodide ion must lie somewhere in this system. This follows from the observation that iodide ion prevents the formation of organically-bound iodine, and MIT is the first substance, containing organically-bound iodine that is formed in the gland. With this in mind it is obvious that if the enzyme system could be obtained in iodide-free state, then it would constitute an excellent model with which to study the quantitative aspects of iodide inhibition. It has been found possible to prepare the enzyme system free of iodide ion and it has been used in quantitative studies similar to those carried out in the intact rat by Wollman and Scow. The results of this work have been accepted for publication

by the journal "Endocrinology" and are in press at the moment (5). A copy of the manuscript is attached to this report.

The results of work with the enzyme system led to the conclusion that the triiodide mechanism accounted remarkably well for the degree of inhibition observed over a wide range of iodide concentrations. While this evidence argues for the triiodide mechanism, one has to proceed with caution since it is not completely certain that the enzyme system is a valid model for events occurring in the thyroid gland, and it must be realized that Wollman and Scow were working with the intact gland in situ in the animal body. However, a close examination of the data of Wollman and Scow shows that they have made an error in interpretation of the data obtained from their tracer studies. The nature of this error, which is peculiar to studies involving isotopically labeled substances, will not be discussed here since it is discussed in some detail in the manuscript attached to this report. When allowances are made for this error in interpretation, it is found that the extent of inhibition observed by Wollman and Scow agrees remarkably well with both the inhibition observed with the isolated enzyme system and that predicted by the triiodide mechanism. This work not only strengthens the theory that iodide acts as an antithyroid via the triiodide mechanism, but it also provides evidence that observations with the isolated enzyme system can be correlated with events occurring in the intact thyroid gland, and that the enzyme mechanism may well be responsible for events observed in the gland.

The Relationship Between the Soluble and Insoluble Enzyme Systems

The work on the relationship between the Fawcett-Kirkwood system (hereafter referred to as the "soluble" system) and the Chaikoff mitochondrial system (hereafter referred to as the "insoluble" system) has not as yet been published.

In the only publication on the insoluble system that has yet appeared (2) two main differences are claimed that distinguish the insoluble system from the soluble one. These are: first, the MIT produced by the insoluble system is protein-bound; and second, the insoluble system occurs only in thyroid tissue. It was reported some time ago (1) that the soluble system occurred in high concentrations in salivary gland tissue and it was postulated that these organs might function in the extrathyroidal metabolism of organically-bound iodine. Since, in addition to thyroid tissue, the Chaikoff group investigated only kidney and liver tissue, we undertook to investigate the occurrence of both enzyme systems in a wide variety of tissues. The results of this investigation are shown in Table 1. It is evident from an examination of these data that the insoluble system occurs in tissues other than the thyroid. It occurs in

four extrathyroidal sites in readily appreciable concentrations. These are: salivary gland, extraorbital lacrimal gland, spleen and stomach. Mitochondrial preparations from thymus, lymph node, small intestine and lung all produce small, but definitely detectable, amounts of protein-bound MIT. We have been able to confirm Chaikoff's observation that mitochondrial preparations from liver and kidney do not synthesize MIT and have extended these observations to include brain skeletal muscle and heart muscle.

While it is quite obvious that the claim advanced by the Chaikoff group, namely, that the insoluble system described by them is confined to thyroid tissue, is not correct, the function of the insoluble system remains obscure. Its relationship, if any, to the soluble system is also not evident. Although it occurs in appreciable concentrations in the tissues which contain the soluble system, it also occurs in two tissues, stomach and spleen, that do not contain appreciable concentrations of the soluble system. The trace activities for the insoluble system detected in thymus, lymph node, small intestine and lung may well have no significance. Mitochondria are the sites of location of many oxidizing enzymes and the small amounts of protein-bound MIT formed in these tissues may well be due to the non-enzymatic iodination of protein by elemental iodine generated by these oxidizing systems.

One feature of the insoluble system in salivary gland tissue merits comment. Chromatographic investigation of mitochondrial preparations from the submaxillary salivary gland that have been incubated with radioiodine show that only part of the MIT formed is protein-bound. About twenty per cent of the total MIT is in the free form. It has been shown that this free MIT has its origin in the mitochondria. By repeated washings it is possible to prepare these mitochondrial preparations free of soluble MIT. If they are then incubated for a period of several hours with buffer, free MIT again appears in the supernatant fluid. This observation indicates that in salivary gland mitochondrial preparations there is either a parallel synthesis of both free and bound MIT or, the protein-bound MIT is slowly hydrolyzed to liberate the free amino acid.

While it is certainly too early to draw any firm conclusions as to the relationship, if any, between the soluble enzyme system of Fawcett and Kirkwood and the mitochondrial system of Chaikoff et al, several points invite speculation. An examination of Table 1 shows that, in general, the two systems occur side by side. Although their concentrations in the same tissue are not comparable, it is noteworthy that all tissues that contain measurable activity for the

soluble system also show activity for the mitochondrial system. The converse is also very nearly true. Only the spleen, among all the tissues examined, shows an appreciable concentration of the insoluble system without, at the same time, having a detectable amount of the soluble one. These facts indicate that there is some connection between the two systems. One possible link is that the soluble system results from the mitochondrial system passing into solution. Many mitochondrial enzymes show this behaviour and there is a great variation in the ease of solubilization. If further investigations should prove this to be the case, then the observations of the California and McMaster laboratories are not in conflict.

The presence of the Chaikoff system in extrathyroidal sites effectively disposes of the implication of the California group that the soluble system is an "artifact" (2). While it cannot be taken as an established fact that either system has a physiological function, it would be rather surprising if such striking catalytic activities were present in the various tissues by mere chance. In the writer's opinion the most recent work of the California group supports the concept that there is some form of iodine metabolism proceeding in the peripheral tissues and the work in the two laboratories seems to be leading to similar conclusions in this respect ¹.

If the presence of these two enzyme systems in the extrathyroidal sites is taken to indicate that there is an extrathyroidal metabolism of iodine, then it is obvious that the submaxillary salivary gland is a major site of this function. Recently Muhler and Shafer, at the University of Indiana, have claimed that the feeding of thyroid substance causes a decrease in the incidence of dental caries in rats on a cariogenic diet (6). Further, they were able to demonstrate that the feeding of an antithyroid substance caused an increase in the incidence of caries. In a very recent paper (7) they report on the effect of hypophysectomy on the incidence of dental caries and on the histology of the submaxillary gland. They draw attention to the fact that their own work, together with that of other people, indicates quite plainly that the submaxillary gland shows marked histological changes under the influence of androgens, estrogens and the thyroid hormone. The most profound changes are produced by hypophysectomy, as would be expected if the changes

* Information has recently reached us, by word of mouth, that the Chaikoff group has independently observed their enzyme system in spleen (Endocrinology, January issue). The writer has not seen the original article as yet and therefore cannot comment further.

observed were due to upsets in hormonal balance. Muhler and Shafer have been able to correlate these histological changes in the submaxillary salivary gland with the incidence of dental caries. They conclude that the influence of these hormones on the incidence of dental caries is mediated through their action on the salivary glands. It is of some interest to note that two independent lines of work indicate an involvement of the submaxillary salivary glands in peripheral hormone metabolism. This may explain the rampant dental caries picture in the hypothyroid human (8).

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8. Becks, H., J. California State Dental Assoc., 26: 1, 1950.

Table 1

<u>Tissue</u>	<u>Enzyme Activity Present</u>	
	Soluble system	Mitochondrial system
THYROID		
Rat	+++	NI
Beef	++	+++
Sheep	NI	+++
Human	+++	NI
SUBMAXILLARY SALIVARY		
Rat	+++	++
Beef	+++	+
Guinea pig	++++	NI
INTRAORBITAL LACRIMAL		
Rat	++	NI
Guinea pig	+	NI
Human	++	NI
Extraorbital Lacrimal (rat)	+++	+++
Stomach (rat)	+	++
Thymus (rat)	-	+
Spleen (rat)	-	++
Lymph node (rat)	NI	+
Lymph node (guinea pig)	-	+
Small intestine	NI	+
Lung	NI	+

The following rat tissues were completely devoid of activity for both systems: liver, kidney, brain, skeletal muscle, heart muscle.

The scale of enzyme activity was based on the per cent incorporation of radioiodine into moniodotyrosine, in excess of that which takes place in boiled controls. For the soluble system a 5% homogenate of the appropriate tissue was incubated for 0.5 hr. under the conditions described in reference 2. For the mitochondrial system the conditions used in reference 3 were followed.

The scale of activities is:	0 - 2% incorporation	-
	2 - 10% incorporation	+
	10 - 25% incorporation	++
	25 - 50% incorporation	+++
	50% + incorporation	++++
	Tissue not investigated	NI

APPENDIX L

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: A comparative study of palatal height, width and length in ancient and modern crania.

Grantee: Dr. D.J.E. Mitchell,
University of Toronto

Project No.: DR-56

Amount of Grant: Nil

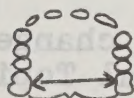
Methodology:

Measurements of the palate were taken in the following manner:

(a) Width: the shorter measurement between the upper first permanent molars measured horizontally at the lingual alveolar crest.

(b) Length: measured from a point midway between the central incisors on a tangent to the lingual alveolar crest, and the central point of a line tangent to the posterior notches of the hard palate.

(c) Height: measured from a perpendicular extended superiorly from the horizontal width measurement, to the greatest height of the median palatal suture.

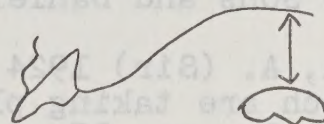


(a)



(b)

Sketch



(c)

The measurements were carefully taken three times on each specimen and the means recorded.

Sample:

1262 specimens were measured involving eighteen ethnic groups. These were examined in the following locations:

The Wistar Institute, Philadelphia, Pa.

The Peabody Museum, Boston, Mass.

The Museum of Natural History, New York, N.Y.

The Smithsonian Institution, Washington, D.C.

An attempt was made to definitely establish sex and wherever possible approximately the age of the specimen. The latter was often extremely difficult due to the lack of specificity in the catalogues. In many instances artefacts were present which eliminated many of the crania.

Collection of the majority of the crude data was completed during the latter part of 1953 and 1954. During the year 1955 the following progress was made.

Statistical Evaluation:

The crude data was subjected to several simple tests to determine the validity of the sample size and sex distribution. It was found that the sample size was adequate for certain races and inadequate for others. The sex distribution was uneven.

Bibliographical Investigation:

The following works were consulted to obtain pertinent information relative to the sampling and methodology:

- Martin, R. Lehrbuch der Anthropologie. Jena, 1928.
- Riesenfeld, Alphonse, Le Palais osseux et ses rapports avec quelques autres caracteres du crâne, Archives Suisses d'Anthropologie General, Geneve Imprimerie Albert Kundig, 1947.
- Shaw, J.C. Middleton., The teeth and bony palate and the mandible in Bantu Races of South Africa, London, John Bole and Sons and Danielson Ltd., 1931.
- Keith, A. (Sir) 1924 Concerning certain structural changes which are taking place in our jaws and teeth, Oral Topics, vol. lv. no. 37.
- Weidenreich, Franz, Apes, Giants and Men. University of Chicago Press, Chicago, Illinois, 1946.
- Hrdlicka, A., Practical Anthropometry. Phila., Wistar Inst.

Associated Investigation:

Since the write is associated with the Research Institute, Hospital for Sick Children, Toronto, and actively engaged in the orthodontic treatment of cleft palate disabilities it was possible to carry out additional studies of the abnormal palatal form therein associated. The methodology chiefly concerns the measurement of identically orientated cephalometric radiographs exposed at six month intervals.

Future Plans:

It is the intention of the writer to augment the amount of crude data already obtained by further examination of other

collections.

It was felt that greater attention should be paid to the racial composition of the samples and the approximate age in spite of the fact that this information is difficult to obtain by mailed request. The majority of the collections examined have not been in active use for many years and the catalogues are frequently not accurate.

Project No.: DS-55

Amount of Grant: \$11.

Material for the study was taken from the Meredith-Higley Longitudinal Anthropometrical Study of children at the University of Iowa. This study consists of serial measurements taken of a group of normal children, mostly of North European ancestry. Measurements of each child begin at 4 years of age and are repeated semi-annually through to the age of 12 years. (As of 1954) Statural, and cephalic and other anatomic dimensions are all recorded, along with dental casts and cephalostatic profile and antero-posterior Roentgenograms.

As my study is to determine whether any co-relation exists between statural increment and antero-posterior growth of the maxilla, I concentrated on the annual statural measurements and took measurements of the length of the maxilla between the subspinale and the Pterygo maxillary fissure from the cephalostatic profile films.

Out of the total number of individuals in the study, I selected 29 boys and 33 girls, these representing the total number available presenting x-rays of sufficient quality to yield accurate measurements.

Analysis of this data will not require many more hours to complete. The thesis, however, will take some time.

APPENDIX M

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: A study of maxillary growth as related to statural growth.

Grantee: Dr. M.R. Culbert,
University of Toronto

Project No.: DR-55

Amount of Grant: Nil.

Material for the study was taken from the Meredith-Higley Longitudinal Anthropometrical Study of children at the University of Iowa. This study consists of serial measurements taken of a group of normal children, mostly of North European ancestry. Measurements of each child begin at 4 years of age and are repeated semi-annually through to the age of 12 years. (As of 1954) Statural, and cephalic and other anatomic dimensions are all recorded, along with dental casts and cephalostatic profile and antero-posterior Roentgenograms.

As my study is to determine whether any co-relation exists between statural increment and antero-posterior growth of the maxilla, I concentrated on the annual statural measurements and took measurements of the length of the maxilla between the subspinale and the Pterygo maxillary fissure from the cephalostatic profile films.

Out of the total number of individuals in the study, I selected 29 boys and 33 girls, these representing the total number available presenting x-rays of sufficient quality to yield accurate measurements.

Analysis of this data will not require many more hours to complete. The thesis, however, will take some time.

I. Sample

The sample for this study originated in 1953 with a group of forty Toronto children aged 5 1/2 - 7 1/2, evenly distributed as to sex.

The sample last year consisted of 34 of the original sample, consisting of 19 boys and 15 girls, with an age range of 6 1/2 - 8 1/2. The study is now entering its third year with approximately thirty of the original sample available.

APPENDIX N

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Continuing studies in electromyographic techniques.

Grantee: Dr. D. H. Jenkins,
University of Toronto

Project No.: DR-37

Amount of Grant: \$2,350.

SUMMARY OF WORK

The original serial study on normal children of electromyographic techniques has been expanded now to include several other aspects of electromyographic technique. The study at present includes the following projects:

Part I (a) The continuation of the serial study on a group of forty Toronto children who originally possessed normal occlusions.

(b) A study of facial asymmetry of the same children employing antero-posterior films.

Part II The evaluation of the practicability and costs of simple methods of treatment of a very prevalent orthodontic condition classified under Angle Class II or post normal mandible utilizing myofunctional therapy.

Part III A project to study the muscle patterns of the dorsal and ventral portions of the temporal muscle in Class II, Division I cases, and Class II, Division II cases in selected mandibular positions.

I. Sample

The sample for this study originated in 1953 with a group of forty Toronto children aged $5\frac{1}{2}$ - $7\frac{1}{2}$, evenly distributed as to sex.

The sample last year consisted of 34 of the original sample consisting of 19 boys and 15 girls, with an age range of $6\frac{1}{2}$ - $8\frac{1}{2}$. The study is now entering its third year with approximately thirty of the original sample available.

Method

(a) Records consisting of:

- (i) Plaster models of the teeth
- (ii) Lateral and antero-posterior cephalometric radiographs in centric, rest and wide open positions of the mandible.
- (iii) Electromyographic tracings of the temporal muscle at rest and in selected actions

are available for 37 of the original 40 children for two years.

The sample is now being collected for the third year and the details of the technique of recording procedure are being perfected.

(b) The plaster models for the two years have been assessed for change in the classical picture of normal occlusal relationships. Change from a normal occlusion to Class II occlusion was noted in five children.

(c) Electromyographic were classified as to:

- (i) Marked activity and inactivity of the posterior temporal muscle in physiological position of the mandible.
- (ii) Active temporal muscles were further classified as to whether this activity was bilateral or unilateral.

The activity patterns obtained were compared with those obtained from the previous year, and an attempt made to correlate changes in muscle pattern with changes in occlusion.

(d) Cephalometric radiographs of the past years.

Sample has been surveyed in cross-sectional studies to:

- (i) Determine the range of facial asymmetry in two planes of space in normal eight year old Canadian children.
- (ii) Determine the normal range of variation of the anatomical structures of the face projected on to two planes of space - the horizontal and the vertical - parallel and at right angles to the palate.
- (iii) The facial structure of the normal children who showed marked activity of the temporal muscle was compared statistically with that of the children showing inactivity of the temporal muscle to de-

termine whether activity of the temporal muscle was associated with facial deficiency and if so, the precise location and dimensional orientation in the vertical and horizontal plane of such deformity.

- (iv) The facial structure of the children who had changed from normal occlusion to Class II Division I was compared statistically with that of the normal children to determine the location and dimensional orientation of any skeletal abnormality if present.
- (v) The facial pattern of each individual who had changed to Class II Division I was superimposed upon a normal variation diagram of the normal sample to study it individually.

II. Sample

Thirteen children exhibiting Class II, Division I malocclusions were selected to investigate the practicability and cost of simple methods of treatment employing myofunctional therapy utilizing the Norwegian monobloc type of appliance.

Method

(a) Pretreatment records consisting of:

- (i) plaster models
- (ii) profile and full face photographs
- (iii) lateral cephalograms in centric, rest and open position of the mandible.
- (iv) temporo mandibular joint x-rays
- (v) electromyograms of the temporal muscle

have been collected for each individual.

(b) Monoblocs were made and inserted on a basis of 12 hours wear per 24 hours, worn at night. Patients are seen at two to four week intervals for simple appliance adjustment.

(c) Preparations are being made to obtain mid-treatment records in order to assess:

- (i) whether tooth repositioning within the jaws is occurring.
- (ii) whether jaw repositioning is occurring.
- (iii) temporo-mandibular joint changes.
- (iv) muscle activity change in the temporal muscle.

III. Sample

Twenty Class II, Division I cases and 10 Class II, Division II cases have been selected to study the muscle patterns of the dorsal and ventral portions of the temporal muscle in Class II, Division I cases and Class II, Division II cases. A control group of twenty children having good occlusion has also been selected.

Method

(a) Preliminary experiments on improvements in electromyographic techniques have been carried out. Bipolar electrodes were used in conjunction with a Grass Model 3-D electroencephalograph.

(b) Tracings were made with the mandible at rest and in selected mandibular positions.

(c) Tracings are being analyzed at present to see if there is any significant difference in the two parts of the muscle in the two groups.

(d) The study was attempted on a group of four year old children. Problems of child management at this age rendered the study impractical.

FINDINGS

on the past year's work are being assembled for thesis publication and will be available within several months. Several points of interest are noted.

1. Unilateral activity of the temporal muscle was noted in a number of active muscles. This has not been reported previously.
2. Activity of the temporal muscle does not appear to be associated with facial abnormality in either the horizontal or vertical plane of space in normal eight year old boys and girls.
3. No abnormality in skeletal structure of the face could be detected in children showing a bite change to Class II at age $6\frac{1}{2}$ - $8\frac{1}{2}$. The facial structure of these children was very favourable. Change at this age was due to dental rather than skeletal change at this age.

PROGRESS REPORTPart I - An electrolyographic study of the temporal muscle
(student assistant - M. Johnson)

This investigation consists of the continuation of a serial study on a group of approximately 30 Toronto children with normal occlusion. The study is now in its third year and the children 7 - 9 year of age at the present time. The records are to consist of study models, cephalometric radiographs and electromyographic tracings.

To date, electrolyographic records have been taken of about 12 individuals to test the method of study. A Grass III D electroencephalograph acceptable to electromyographic work was employed. An evaluation of the different types of surface electrodes was made as also the various electrolytes, dermal adhesives, etc. which may be used in their application. Comparisons were made of several recording techniques which involved different combinations and positions of recording, reference and ground electrodes.

These preliminary recordings were taken of subjects with both normal and abnormal occlusion. The recording procedure used previously for this serial group were repeated and then alterations made to investigate possibilities of further standardization to permit more accurate comparison from year to year.

The sample is now being collected and the details of the recording procedure worked out.

Part II - An evaluation of the monobloc appliance in treatment of Class II malocclusions (orthodontist assistant - Dr. A. Jarvis.)

Purpose: To evaluate the practicability and cost of simple methods of treatment of the very prevalent orthodontic conditions classified under Angle Class II or post normal mandible, utilizing the Norwegian monobloc type of appliance.

Method: Records consisting of

- (a) plaster models
- (b) profile and full face photographs
- (c) lateral cephalograms in centric, rest and open position of mandible
- (d) temporo-mandibular joint x-rays
- (e) electromyograms of the temporal muscle

were made on the thirteen Angle Class II children selected as

subjects before treatment was commenced. These records will be compared with similar records taken when treatment is completed in order to assess:

- (a) whether changes occurring during treatment are due to tooth movement or change in the facial skeletal growth pattern of the child;
- (b) whether marked changes in profile may be produced by simple, conservative therapy in young children.

Progress: Treatment is progressing satisfactorily with the usual mishaps encountered in such treatment. Three monoblocs have had to be replaced due to loss. One patient has moved from the district. Since the study has been in progress nearly a year now, preparations are being made to obtain mid-treatment progress records.

Part III - An electromyographic study of the temporal muscle in Class II Cases, using bipolar surface electrodes (student assistant - Dr. R. Boyko)

Purpose: To study the muscle patterns of the dorsal and ventral portions of the temporal muscle in Class II, Division I cases and Class II, Division II cases in the following mandibular positions:

- (1) rest
- (2) jaw left movements were made to
- (3) jaw right the extreme lateral pos-
- (4) maximal mouth opening itions
- (5) forced protrusion
- (6) biting end to end in
- (7) centric occlusion isometric
- contraction

Method: Preliminary experiments were carried out with different types of electrodes, electrolytes, and dermal adhesives. The decision to use bipolar electrodes was reached after comparisons were made of several recording techniques using different positions for ground and reference electrodes. The Brass model 3-D electroencephalograph which can be used for electromyography also was moved to the Burlington Research Centre for a period of seven weeks. The sample consisted of thirty children 10 - 13 years old from the control group and

was composed of 20 Class II Division I cases and 10 Class II Division II cases. Recordings were not taken until the skin resistance was lowered to the vicinity of 3000 ohms.

The tracings are being analyzed to see if there is any significant difference in the two parts of the muscle in the two groups. These findings are to be compared with other studies.

For a control a similar study was carried out on a group of twenty children having good occlusion by Dr. Latiff from the Department of Anatomy.

Originally this study was to be performed on normal 4 year old children from the Burlington serial group. Attempts to reduce the procedure to a reasonable length of time for children of this age failed. Two types of head-holders were devised to facilitate electrode placement but movement artifacts could not be controlled with them.

Studies have been made to the effect of various acids gives satisfactory results. In this section, analysis is carried out with potassium hydroxide; 100 ml of 1% solution is obtained in the distillation step from a total of 100 ml volume of sample, water and sulfuric acid; the pyramidal 2 ml acid and the concentration of strength will be 1% sulfuric acid. In the reagent (or indicator) have been checked.

The analyses of teeth from 15 children have been included.

APPENDIX O

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1956

Subject: Methods for destroying the organic matter in teeth, bones, etc. prior to determination of fluorine.

Grantee: Dr. O. J. Walker
University of Alberta

Project No.: DR-28

Amount of Grant: \$900.

SUMMARY OF WORK

Further studies have been carried out on the destruction of organic matter, distillation with sulfuric acid and stability of the zirconyl-sodium alizarin sulfonate reagent used for the photoelectric colorimeter determination. These studies have led to the adoption of procedure which gives satisfactory results. In this method, fusion is carried out with potassium hydroxide; 100 ml of distillate is obtained in the distillation step from a total of 150 ml volume of sample, water and sulfuric acid; the normality of the acid and the concentration of zirconyl salt and sodium alizarin sulfonate in the reagent (or indicator) have been changed.

The analyses of teeth from 19 Eskimos have been included.

Scott's reagent was made up so that it was 2.7N with respect to HCl and H_2SO_4 and contained 0.301 gm. zirconyl chloride and 0.07 gm. of sodium alizarin sulfonate per litre. Twenty different modifications of this were studied in which the normality with respect to the acids varied from 2.7 to 3 N, the zirconyl chloride from 0.258 to 0.387 gm. per litre and the sodium alizarin sulfonate from 0.04 to 0.09 gm. per litre. Two of the reagents investigated yielded satisfactory standard transmission - composition curves over an extended fluoride concentration range.

Some of the results are given in Table 1. In this table amounts of fluoride are based on using known amounts of fluoride in 100 ml. samples of water. The absolute amounts of fluoride in milligrams can be obtained by multiplying per cent fluoride by the factor 0.1. Values in the second column (2) are for the reagents as specified by Scott. In column 3 (3) the reagent

PROGRESS REPORT

Further work on this problem was carried out in the summer of 1955 with the assistance of J. F. Hanlan who has a B.Sc. from the University of Alberta with First Class Honors in Chemistry.

The investigations undertaken consist of extensions to those reported on the project in September 1953.

Experimental work may be divided into four divisions:

- (1) A further study of the method of analysis for fluoride;
 - (2) Extensions to methods of fusion of organic materials;
 - (3) Critical examination of the recovery of fluoride in the distillation step;
 - (4) The determination of the fluoride content of some Eskimo teeth.
- Most of the time was spent on the third part as it was here that results were not conclusive when earlier reports were submitted.

1. Methods of Analysis

Methods of analyzing for fluorine are essentially the same as outlined in the previous reports. They are based on Scott's (1) modification of the zirconyl-sodium alizarin sulfonate reagents adapted for the Lumetron photoelectric colorimeter as previously described. The method as described by Scott is satisfactory for determining from 0 to 0.15 mg. of fluorine. It was thought that if the reagents were mixed in different proportions it might be possible to extend the range and make the method applicable to large amounts of fluorine and at the same time be useful for very small amounts as well.

Scott's reagent was made up so that it was 2.7N with respect to HCl and H₂SO₄ and contained 0.301 gm. zirconyl chloride and 0.07 gm. of sodium alizarin sulfonate per litre. Twenty different modifications of this were studied in which the normality with respect to the acids varied from 2.7 to 3 N, the zirconyl chloride from 0.258 to 0.387 gm. per litre and the sodium alizarin sulfonate from 0.06 to 0.09 gm. per litre. Two of the reagents investigated yielded satisfactory standard transmission - composition curves over an extended fluorine concentration range.

Some of the results are given in Table 1. In this Table amounts of fluoride are based on using known amounts of fluoride in 100 ml. samples of water. The absolute amounts of fluoride in milligrams can be obtained by multiplying parts per million by the factor 0.1. Values in the second column (A) are for the reagents as specified by Scott. In column 3 (#19) the reagent

is 2.7 N acid 0.366 gm. of zirconyl chloride and 0.085 gm. sodium alizarin sulfonate and in column 4 (#12) the solution is 3 N acid containing 0.344 zirconyl chloride and 0.08 gm. sodium alizarin sulfonate per litre.

Table 1

ppm F	% Transmission		
	A	#19	#12
0	45.4	38.2	39.0
0.2	47.1	42.1	42.9
0.4	50.0	46.7	48.4
0.6	52.9	52.9	53.8
0.8	56.5	60.1	61.4
1.0	61.1	67.6	67.8
1.2	65.5	74.3	76.2
1.4	70.0	81.1	81.8
1.6	-	85.5	87.5
1.8	-	89.8	91.2
2.0	-	91.4	94.4
2.2	-	94.5	-

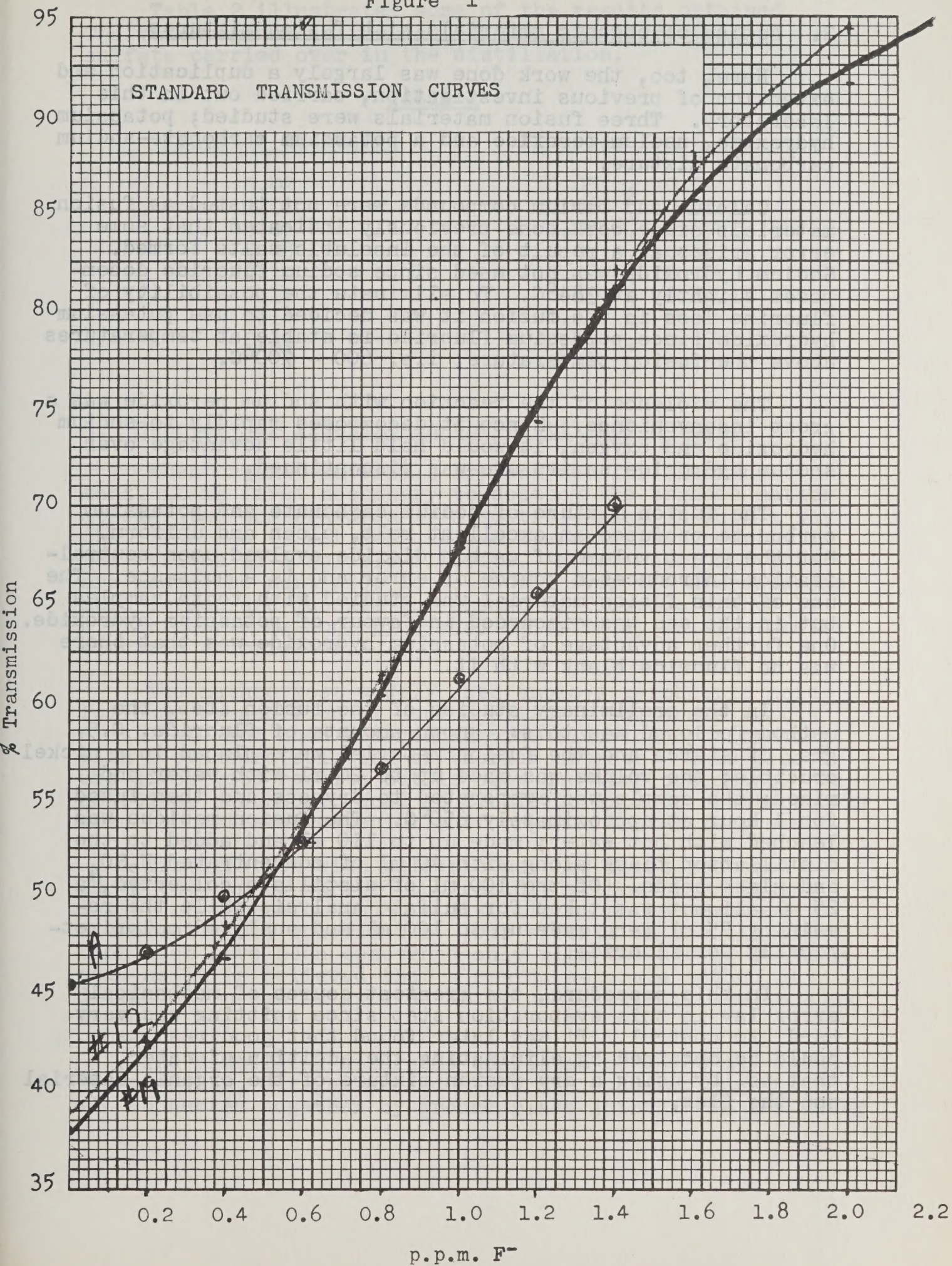
Figure 1 contains the graphs obtained when the data in Table 1 are plotted, - per cent transmission vs. ppm fluoride.

Both #12 and #19 reagents yield curves with satisfactory slopes over an extended range. One difficulty met with in earlier work was that calibration curves taken over a period of time with the same indicator were not the same, indicating that some internal changes were taking place. In our studies with #19 it was found that it was very stable over a period of at least three months. It was used in preference to other indicators and results for the analysis of teeth in Section 4 were obtained with it.

Another factor studied was the effect of temperature on the measurement of fluorine. By working at different temperatures it was found that results varied considerably. We came to the conclusion that all determinations should be carried out within 2°C of that at which the indicator was standardized.

We have again established the fact that every new batch of indicator must be standardized even though it was made up in the same concentrations as that of an earlier one.

Figure 1



2. Ashing of organic materials with fusion mixtures

Here, too, the work done was largely a duplication and extension of previous investigation, carried out in this laboratory. Three fusion materials were studied; potassium hydroxide, sodium peroxide and a potassium carbonate-sodium carbonate mixture.

Calcium and barium compounds were not tested as fusion materials since work done previously indicated that some error arises as a result of the insoluble salts formed. Sodium hydroxide was not used since sodium fluoride decomposes slightly at 384°C . To eliminate the possibility of fluorine loss in the fusion it was decided to use potassium hydroxide since potassium fluoride is stable at temperatures above the fusion temperature, i.e. $600 - 700^{\circ}\text{C}$.

The violence of the reaction with sodium peroxide was a great inconvenience. Since it decomposes rapidly to sodium hydroxide and oxygen, it would hold little advantage over sodium hydroxide so its use was discontinued.

The fusion mixture of sodium carbonate and potassium carbonate produced an excellent melt, clean and uniform. But the great volume of carbon dioxide evolved upon neutralization introduces a source of error and is a nuisance. The use of this fusion material was studied with tooth samples but in the end was discarded in favour of potassium hydroxide. One further advantage of potassium hydroxide was that there was no fluoride blank with it.

In the preliminary studies of the fusion step, the method followed was this: known amounts of fluoride, 0.5 gms. of starch and the fusion material were placed in a nickel crucible; the volume was made up to 20 ml with water and mixed; the sample was evaporated to dryness and then fused for 1 hour at approximately 650°C . The fusion residue was leached with hot water; made up to 110 ml and added to the distillation flask along with 40 ml of concentrated H_2SO_4 and glass beads. Of the 100 ml of distillate collected, a 25 ml aliquot was taken for sulfate analysis while the remaining 75 ml were made up to 100 ml and analyzed colorimetrically for fluoride.

In this procedure, the greatest source of mechanical error lay in this evaporation step since spitting was very hard to control. It was later found that good results could be obtained by eliminating the addition of water, that is, by using a dry fusion mixture of the organic material and the flux.

Table 2 illustrates some of the results obtained. The values given in the last column are corrected for sulfate carried over in the distillation.

Table 2

<u>Fluoride added,mg.</u>	<u>Fluoride found, mg.</u>
0.02	0.05 , 0.04
0.04	0.06 , 0.06
0.06	0.07 , 0.07
0.08	0.07 , 0.08
0.10	0.08 , 0.09
0.12	0.10 , 0.10
0.14	0.12 , 0.12
0.16	0.15 , 0.17
0.18	0.18 , 0.18

3. Recovery of fluorine in the distillation step.

When organic materials are analyzed for fluorine content, it is first necessary to destroy the organic matter at elevated temperatures by a suitable flux in such a way that fluorine is not lost in the operation but remains in the residue as a soluble fluoride. After water and acid are added the next step is to distil over the hydrofluoric acid formed and measure the amount of fluoride either by a titrimetric method, by visual colorimetry or by a photoelectric colorimeter.

The method most widely used employs perchloric acid followed by addition of sodium alizarin sulfonate to the distillate and then determining the fluoride by titrating with a standard solution of thorium nitrate. This procedure gives good results but is slow and time consuming. In addition, perchloric acid is liable to explode spontaneously when concentrated especially if organic matter is present.

Distillation with sulfuric acid as first proposed by Thrun (2) can be carried out rapidly without introducing danger of explosion and the distillate can be examined in a photoelectric colorimeter. Our efforts on the distillation step have been concerned only with the use of sulfuric acid as it is believed that a rapid, accurate method of distillation can be evolved using comparatively simple apparatus.

The distilling apparatus consisted of a 500 ml. round bottom flask, a right angled elbow and a 50 cm. condenser all connected together by ground glass joints. In the flask

were placed 110 ml. of the water solution containing the fluoride, 40 ml. of concentrated sulfuric acid and some glass beads to prevent bumping. The flask was heated with a Meker burner and the required amount of distillate was obtained in twelve to fifteen minutes.

The distillate should meet these requirements: it should contain all of the fluoride in the original sample; it should be low in sulfate as sulfate has a bleaching action on the zirconyl chloride - sodium alizarin sulfonate lake similar, but to a less degree, to that of the fluoride ion; the volume of distillate should be as small as possible. Corrections have been worked out in this laboratory in previous investigations for the effect of varying amounts of sulfates and these have always been applied. This necessitates sulfate determinations on an aliquot of each distillate which is done by titrating by a standard solution of barium chloride using THQ as the indicator. In some of the experiments carried out early in the summer, sulfate concentrations were in the 500 to 1000 part per million range. Corrections needed for sulfate content were thus of considerable magnitude. It was found, however, that a plug of glass wool placed in the elbow of the distillation apparatus reduced the concentration of sulfate in subsequent distillations to 100 to 200 p.p.m. requiring little or no correction. This modification allowed us to increase the volume of distillate to 100 ml and thus assured us of a better recovery of fluoride.

Investigation of the boiling points of sulfuric acid - water mixtures showed that in the distillation outlined above, the last 40 ml of distillate were distilled at or above the temperature recommended by other workers for complete fluorine recovery. It was also found that with 110 ml of sample at least 20 ml of concentrated sulfuric acid were required for complete recovery in the middle concentration. So to ensure completeness on the upper ranges, 40 ml continued to be the volume of sulfuric acid used.

A great many series of distillations were carried out in which the effect of time of distillation, sulfate concentration in distillate, volume of distillate and completeness of fluorine recovery were studied. Table 3 represents one of the series of distillations. The column, fluoride obtained, has been corrected for sulfate. From this and other series it may be concluded that distillation with sulfuric acid gives good recovery of fluoride in a comparatively short distillation time.

Table 3

Sample #	F ⁻ added	Distillation time	F ⁻ found
1	0 p.p.m.	21.39"	0.1 p.p.m.
2	0	20.52	0.1
3	0.2	16.23	0.2
4	0.2	17.56	0.4
5	0.4	22.25	0.4
6	0.4	17.35	0.4
7	0.6	15.39	0.4
8	0.6	17.55	0.5
9	0.8	19.27	0.7
10	0.8	19.50	0.8
11	1.0	19.42	0.8
12	1.0	16.58	0.9
13	1.2	17.19	1.0
14	1.2	16.08	1.3
15	1.4	13.40	1.2
16	1.4	14.58	1.2
17	1.6	15.39	1.5
18	1.6	15.53	1.7
19	1.8	12.10	1.8
20	1.8	14.30	1.8

4. Analysis of teeth

With the completion of the studies on methods of analysis, ashing techniques and distillation procedures, making use of materials containing known amounts of fluoride, it was decided to use the method on the analysis of teeth.

To gain experience, a number of teeth obtained from Dr. H. R. MacLean of the Faculty of Dentistry were ground up together and analyzed in 0.2000 to 0.5000 gm. aliquots. Fifteen results were obtained on a total of 5,700 gm. of teeth. The average value obtained for these was 0.0347%.

Dr. Carley, Dental Health Officer of the Charles Cam-sell Indian Hospital, Indian Health Services, Department of National Health and Welfare, was kind enough to supply us with teeth extracted from about 20 adult Eskimos on a trip to Cambridge Bay and other Arctic Coast communities, April 5 to 28, 1955. He also supplied us with such information as the patient's name, sex, age, type of diet and

whether the particular tooth was carious. However, he did not state whether the tooth was cuspid, bicuspid or otherwise.

Each tooth was given the following preliminary treatment. It was soaked for a day in acetone, then a day in ethyl alcohol, followed by treatment for similar periods, in two portions of distilled water. The tooth was then dried for two days in an oven at 110°C . After weighing, grinding to a fine powder was done with a mortar and pestle. Because of the hardness and brittleness, grinding was difficult as pieces tended to fly out of the mortar. Loss of parts of the sample was prevented by placing the tooth on a copper cylinder in the mortar and using the small end of the pestle to break up the tooth.

The next step was to make a mixture of the tooth with potassium hydroxide, 3 gm. of the latter for each 0.5 gm. of sample. The mixture was then transferred to a nickel crucible and heated in an electric furnace for one hour at 650°C . After cooling, the melt was treated with hot water and brought up to a specific volume depending on the weight of the tooth. Referring to Table 4, the volume used for teeth #1, 3, 4, 5, 6, 11, 13, 15, 16, 18 and 19 was 200 ml; for teeth #9 and 12 was 250 ml; for #7 and 17 was 300 ml; for #8 and 10, 500 ml; and for #2 and 14, 600 ml.

A 100 ml. aliquot for each tooth was used along with 40 ml. of concentrated sulfuric acid and 10 ml. of distilled water in the distillation step. Duplicate determinations were run in all cases. About 100 ml. of distillate were collected in a volumetric flask and made up to the mark.

A 25 ml. aliquot was used for determining sulfates. The remaining 75 ml. was diluted to 100 ml. To this 5 ml. of the zirconyl sodium alizarin sulfate reagent, #19 was added and then allowed to stand for exactly an hour. This was next transferred to the 150 mm. cuvette and the percent transmission measured in the Lumetron photoelectric colorimeter. The value obtained was compared with the standard curve and the uncorrected amount of fluorine obtained. This was then corrected for sulfate. On the basis of using aliquots at different stages in the determination the percentage of fluorine was calculated. Results are shown in Table 4. Column 2 indicates the general condition of the teeth of the patient. Each quantity in column 4 is the average of two determinations.

Table 4

Tooth #	Caries incidence	Weight of tooth	% fluorine
1	-	0.8624 gm.	0.067
2	±	2.8370	0.034
3	+	0.6379	0.088
4	-	0.7511	0.043
5	-	0.9570	0.034
6	-	1.0805	0.024
7	+	1.3860	0.063
8	+	2.1637	0.046
9	+	1.0816	0.043
10	+	2.0498	0.037
11	-	0.8044	0.054
12	-	1.2778	0.038
13	-	0.7810	0.074
14	±	2.6532	0.025
15	±	1.0105	0.050
16	±	0.9058	0.045
17	?	1.3634	0.040
18	-	0.6808	0.062
19	-	0.7946	0.066

The percentages of fluorine obtained are somewhat larger than have been reported by some analysts but are of the same order as those of Armstrong for teeth from high fluorine areas (3). These high values may be due to a diet of fish and seal with some consumption of the bones of these animals. The eating of bone is a common trait of the Eskimos.

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APPENDIX P

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

May 1955

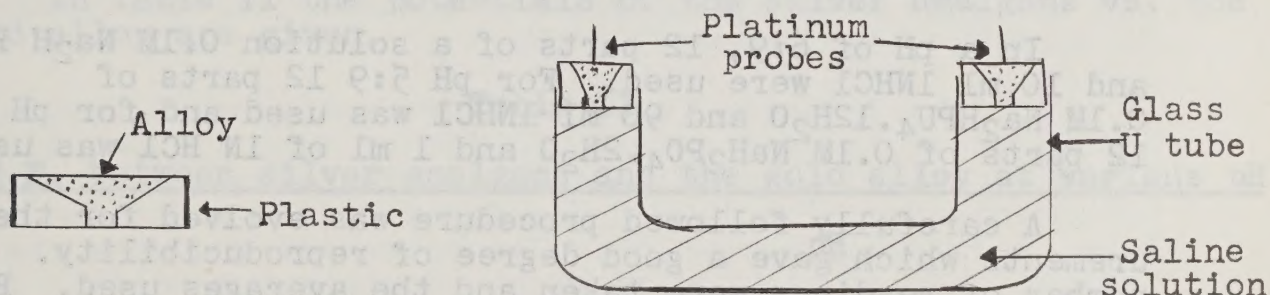
Subject: The electropotentials of dental alloys.
Grantee: Dr. S. G. Davis and Dr. H. R. MacLean,
University of Alberta.
Project No.: DR-43 Amount of Grant: Nil

PROGRESS REPORT

The potentials of several silver amalgams and of a gold alloy were determined when in contact with saline solutions of various pH's. These potentials were measured against a saturated calomel cell and also between the various combinations of the gold alloy and silver amalgams.

The equipment used was as follows. A constant temperature bath was maintained at 37°C and all measurements were made at this temperature.

Commercial amalgams were made into buttons and mounted in a round plastic holder as shown in the diagram.



In making potential measurements the buttons were mounted as shown in a U tube containing a saline solution. This tube was then mounted in the constant temperature bath. Measurements were made with either a saturated calomel electrode or a second amalgam in the other arm of the tube. Platinum probes were used to make contact with the alloys. Measurements were made with the probes dry and touching the amalgam, and the probes immersed in a drop of saline solution and touching the amalgams. As no difference was noted in the two types of measurements, subsequent measurements were made with a dry contact.

The potential was measured by two methods, using a condenser-ballistic galvanometer potential difference meter or by using a vacuum tube electrometer.

The condenser-ballistic-galvanometer potential-difference meter was similar to that described by Schriever and Diamond (J. Dental Res. 31: 20 S, 1952). However it was found necessary to maintain the condenser at a constant temperature ($27 \pm 0.1^\circ \text{C}$) to give a reproducible calibration curve. This method was quite satisfactory.

The vacuum tube electrometer was model #200A obtained from Keithley Instruments, Cleveland, Ohio. It contains a Victoreen 5803 electrometer tube. This instrument requires an extremely small current (approx. 10^{-12} amps.) to operate and so minimizes polarization. Some difficulty was encountered with this instrument as it is very sensitive to electrostatic charges.

In the saline bridge in the cell a Krebs-Ringer solution was used except that CaCl_2 was not included. At the pH's used, $\text{Ca}_2(\text{PO}_4)_3$ precipitated. The solution was

100 parts	0.90% NaCl
4 parts	1.15% KCl
1 part	2.11% KH_2PO_4
1 part	3.82% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$

To this solution 12 parts of buffer was added to prepare three solutions of pH 6:9, 5:9 and 4:1.

In a pH of 6:9, 12 parts of a solution $0.1\text{M Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 10 ml 1N HCl were used. For pH 5:9 12 parts of $0.1\text{M Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$ and 90 ml 1N HCl was used and for pH 4:1 12 parts of $0.1\text{M NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 1 ml of 1N HCl was used.

A carefully followed procedure was evolved for the measurements which gave a good degree of reproducibility. A large number of readings were taken and the averages used. Before each reading the amalgam or alloy surface was polished with a 4/0 polishing paper.

Measurements were made between various combinations of silver amalgams and the gold alloy and between silver amalgams and the saturated calomel electrode. The latter electrode was used to determine absolute potentials for the gold alloy and silver amalgams. Three solutions were used.

The potentials determined are recorded in the following tables.

The absolute E.M.F.'s of the silver amalgams are listed in Table I. These were determined by measuring them against a saturated calomel electrode of -0.244 volts. The alloy is

the negative electrode in this case. A measured E.M.F. + 0.48 would give an amalgam potential of + 0.23.

Table I

Absolute E.M.F. of silver amalgams in volts at various pH

	pH		
Amalgam	6.9	5.9	4.1
Truedentalloy 7 $\frac{1}{2}$ Hg to 5 alloy	+ 0.23	+ 0.20	+ 0.30
Truedentalloy 5Hg to 5 alloy	+ 0.23	+ 0.33	+ 0.29
20th Century alloy	+ 0.23	+ 0.22	+ 0.23
Lang's White Beauty alloy	+ 0.15	-	+ 0.23
Cresilver alloy	+ 0.23	+ 0.25	+ 0.28
Fellowship alloy	+ 0.18	+ 0.18	+ 0.23

These potentials are given a sign according to the American system where Zn Zn^{++} is + 0.76 volts.

In Table II the potentials of the silver amalgams vs. the gold alloy are given.

Table II

E.M.F. between silver amalgams and the gold alloy at various pH

	pH		
	6.9	5.9	4.1
Truedentalloy 7 $\frac{1}{2}$ Hg to 5 alloy	0.48	0.55	0.54
Truedentalloy 5 Hg to 5 alloy	0.49	0.58	0.57
20th Century alloy	0.48	0.57	0.59
Lang's White Beauty alloy	0.40	0.45	0.52
Cresilver alloy	0.48	0.59	0.53
Fellowship alloy	0.43	0.52	0.50

The values listed are the average for several measurements using different buttons made from the same alloy. With these cells the silver amalgam is the negative electrode and the gold

alloy the positive electrode.

From Table I and Table II the absolute E.M.F.'s for the gold alloy may be calculated at different pH's. These calculated values are

pH	E.M.F.
6.9	- 0.25
5.9	-0.35
4.1	- 0.28

The following observations can be made from the above results.

1) The E.M.F. of the cells is rather difficult to measure since surface films can change the potential. Also since the concentration of the metal ion of the alloy being measured is low (almost zero) in the electrolyte the E.M.F. will vary rapidly as the metal ion concentration in solution changes as current flows.

2) pH appears to have little effect on the E.M.F. of the amalgams although there is a tendency for the potential to increase with increasing pH.

3) The potential of the silver amalgams is approximately +0.25 which indicates that the tin or zinc in the alloy is largely controlling the potential. The standard electrode potentials for zinc is +0.76; for tin is +0.14; for copper -0.34 and for silver -0.80. The potential produced then is between that of tin and of zinc. Further information will be sought about the controlling metal by making measurements of E.M.F. using solutions with various Sn^{++} concentrations and with various Zn^{++} concentrations. The ion concentration which affects the E.M.F. most will indicate the metal controlling the E.M.F.

4) The potential of the gold alloy is about - 0.28. Here it appears that the copper in the alloy has quite an influence on the E.M.F. The E.M.F.'s of the other constituents in the alloy are all more negative than -0.28 volts.

5) A potential of the order of 0.5 volts exists between the gold alloys and the silver amalgams. This potential appears to increase slightly with increased pH.

Further Work:

Both Dr. H. R. MacLean and Dr. S. G. Davis are taking sabbatical leave for the following term so that work on this project will be suspended until their return. The project results indicate to date that further work on the metal (or compounds of the metals in the alloys) needs to be done to show what components of the alloys are controlling the E.M.F. Then it might be possible to reduce the amount of these potentials by suitable choice of metals.

The effect of polarization on this E.M.F. must be determined. It is proposed that these aspects of the problem will be investigated in the term of 1956-57.

A quantitative method has been selected, and found sufficiently sensitive to detect hyaluronidase activity in normal human saliva.

Comparative tests are being undertaken on samples of saliva, and gingival pocket secretions from patients with, and without, clinical gingivitis.

Tissue-destructive agents in cell-free extracts of gingival bacteria were demonstrated by Shultz-Bunt, Devar, and Gibby (1953) (1). Consistently present in extracts prepared from inflamed gingiva was a spreading factor (hyaluronidase).

That such a spreading factor could play a part in producing gingivitis was supported by the demonstration that topical applications of hyaluronidase to human gingiva produced tissue changes similar to those found in gingivitis.

In addition, the idea that hyaluronidase is important in gingival destruction was supported by histologic studies which show that the amorphous ground substance in the gingival connective tissue contains hyaluronic acid, and that this is removed in local inflammatory areas. (2)

A study was undertaken to investigate the possible hyaluronidase activity of the four organisms isolated from the mouth of a patient with hypertrophic gingivitis by Macdonald

Holder of a Graduate Dental Research Fellowship (S. G. Davis) during 1954-55 under the supervision of Dr. Frank Graham in the Faculty of Dentistry, University of Toronto.

APPENDIX Q

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

September 1955

Study of Hyaluronidase Activity in Oral Bacteria

by

Clifford Reynolds *

Biochemical investigation of oral bacteria capable of producing a transmissible subcutaneous infection in guinea pigs has shown no demonstrable hyaluronidase activity in these organisms.

A quantitative method has been selected, and found sufficiently sensitive to detect hyaluronidase activity in normal human saliva.

Comparative tests are being undertaken on samples of saliva, and gingival pocket scrapings from patients with, and without, clinical manifestation of gingivitis.

Tissue-destructive agents in cell-free extracts of gingival bacteria were demonstrated by Shultz-Hautd, Dewar, and Bibby (1953) (1). Consistently present in extracts prepared from inflamed gingiva was a spreading factor (hyaluronidase).

That such a spreading factor could play a part in producing gingivitis was supported by the demonstration that topical applications of hyaluronidase on human gingiva produced tissue changes similar to those found in gingivitis.

In addition, the idea that hyaluronidase is important in gingival destruction was supported by histologic studies which show that the amorphous ground substance in the gingival connective tissue contains hyaluronic acid, and that this is removed in local inflammatory areas. (1)

A study was undertaken to investigate the possible hyaluronidase activity of the four organisms isolated from the mouth of a patient with hypertrophic gingivitis by Macdonald

* Holder of a Graduate Dental Research Fellowship (\$3,000) during 1954-55 under the supervision of Dr. Bruce Crocker in the Faculty of Dentistry, University of Toronto.

(1954) (2), namely a gram-positive nonmotile rod (J.R.3), a gram-negative motile rod (K80), and two gram-negative nonmotile rods (K92, and K110). These represent a minimum combination capable of producing a transmissible infection in guinea pigs.

Method and Materials

The first problem was to find a sensitive, reproducible qualitative test for the enzyme. The mucin clot prevention test of D. McClean (3) was chosen, because of its sensitivity. The power of hyaluronic acid to form the mucin clot with serum albumin disappears before any appreciable decrease in its viscosity can be noted, or any chemical test for depolymerization products of hyaluronic acid can be applied.

From supernates of cultures of organisms grown in liquid media, 0.5 ml. samples were incubated at 37°C, at approximately pH7, for sixteen hours with a mixture containing 0.25 ml. of hyaluronic acid solution (0.4mg. per ml.), 0.25 ml. of crystalline serum albumin solution (1%) in physiological saline, and 0.5 ml. of water. After incubation the tubes containing the digests were placed in ice water, and 0.1 ml. of 2N acetic acid was added to each one. The more hyaluronidase activity present, the less clot formation occurred.

The second task was to find a positive hyaluronidase producing control organism which could be used repeatedly, and maintain its activity, and which would grow, and produce the enzyme under the same conditions as were necessary to grow our test cultures. These requirements were met by a strain of hemolytic Group A streptococci.

The third problem has centred around the cultivation of the very fastidious K110; the difficulty here being to grow it in a liquid medium, which would support the growth of our positive control streptococcus, and the production, and detection of hyaluronidase. The only medium which has supported K110 has been Difco Thioglycolate Broth (B236) which contains agar. However, this medium prevented the apparent activity of hyaluronidase, according to the mucin clot prevention test.

Commercial preparations of testicular hyaluronidase were obtained, and these were used to observe the effects of different media and of different enzyme concentrations on the results of mucin clot tests for hyaluronidase activity. Although the testicular enzyme differs from the bacterial hyaluronidase in substrate specificity, they are both active against hyaluronic acid, and it is believed the "hyaluronidase" is a complex of at least two enzymes.

Using approximately 2.5 Turbidity Reducing Units of enzymes it is apparent that the kind of medium does not affect the result. Overnight incubations were used at all times. The clearest tubes, and best observable activity has been obtained with media containing brain heart infusion broth. No difference between controls, and tubes with enzyme was obtained with B236, and agar. The activity is greatly reduced when peptone water, or B236 without agar is used.

Using the medium with brain heart infusion broth, and serial dilution of the enzyme, clear cut activity could be detected down to 0.05 T.R.U. with overnight incubation.

Two types of controls have been used, either uninoculated tubes, or known negative cultures. The latter have been found to give more precipitate on acidification than sterile tubes. One danger is that repeated growth of a negative strain in a medium containing hyaluronic acid will produce a strain which has become active. This has occurred during our experiments.

Findings

J.R.3, K70, and K92 have been repeatedly cultivated in brain heart infusion broth with, and without, hyaluronic acid present in the media. Supernates, of these three organisms, under the conditions of our tests, alone, or combined do not produce a detectable amount of hyaluronidase.

K110 has presented the problem of media suitable for our test. Limited to the media B236 with agar, we have tried fractionation with Ammonium Sulphate and the addition of various strengths of ethylene diamine tetra acetate to our test mixtures in an attempt to remove possible inhibition by ions. Results with these methods used on supernates of the positive control streptococcus strain have not proved satisfactory.

In view of the technical difficulties which we have encountered in the application of the mucin clot test to the determination of hyaluronidase activity, the colorimetric method of Greif (4) was investigated. Preliminary experiments with this method have shown it to be sufficiently sensitive to detect the hyaluronidase activity present in normal human saliva.

Studies now in progress deal with hyaluronidase activity of resting saliva, and scrapings from the mouths of patients with gingivitis as compared to that from normal mouths. Thus, the relationship between this activity, and the occurrence of lesions will be studied, before proceeding further toward an

investigation of the source of the enzyme.

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Appendix R

ORTHODONTIC RESEARCH

by K.J. Paynter

Introduction:

Orthodontics is that branch of dentistry concerned with the treatment of malocclusion. Research in the orthodontic field has sought the underlying anatomical or physiological reasons for the tooth derangement. Such information would thus enable an operator to treat cases of malocclusion more intelligently, or possibly to recognize and intercept early signs of abnormality.

Malocclusions are generally classified according to the method of Angle (189-) who placed individuals into various categories depending on the clinically observed relation of the upper to the lower first permanent molar, on the assumption that these teeth always had a stable relation to the upper and lower jaws respectively. For example, Class I cases have a "normal" relationship of first molars, wherein the mesio-buccal cusp of the upper tooth occludes in the buccal groove of the lower in centric relation. Class II of Distocclusion cases demonstrate a more distal relation than normal of the lower tooth to the upper. Thus the mesio-buccal cusp of the upper tooth fits into the embrasure between the lower molar and second bicuspid. Class III or Mesiocclusion cases have the lower first molar mesial to the normal or classic position. In these cases the mesio-buccal cusp of the upper molar fits into the disto-buccal groove of the lower tooth.

In addition, each class is divided into Divisions, depending on whether or not the anterior teeth are malposed, and further subdivided depending on whether the molar deformity is unilateral or bilateral.

It should be emphasized that this classification groups individuals into various categories solely on the basis of the relation of teeth one to the other, on the erroneous assumption that the first permanent molars always bear the same relationship to the skull or mandible, and it ignores the underlying cause of the derangement. Generally speaking, for research purposes such grouping is made and comparisons are made between the groups. The question naturally arises whether this grouping is reasonable since any of the derangements may result from one or more fundamental causes. Such causes might be incompletely listed thus:

1. Aberrations in skeletal growth from such diverse causes as genetics, endocrine dysfunctions, habits, etc., etc.
2. Malpositioning of teeth within normal jaws from early loss of primary teeth, local habits, etc.

3. Possibly abnormal muscle function.

4. Various combinations of the above.

Orthodontic Research Methods:

Any of the methods employed in research may, of course, be applied to the orthodontic field, but for purposes of this discussion the four methods most commonly used will be elaborated on. These may be listed as:

1. The use of plastic or stone casts of the teeth and alveolar processes.
2. Anthropometric studies.
3. Electromyography.
4. Cephalometrics.

1. Casts: These are of value in determining the relations of the teeth one to the other within the jaw and they give some information on the relation of the teeth to the alveolar process, but they are of little or no value in determining the relation of the teeth to the rest of the jaw and to the skull. Clinically, their chief use is for planning treatment technique and for assessing results.

2. Anthropometrics: Since cases of functional malocclusion are seldom if ever seen in primitive dentitions, it has been felt that by acquiring a more thorough knowledge of the pattern by which primary teeth are replaced by their permanent successors, and the wear patterns on the teeth, etc. one might get a better idea of how nature intended man's dentition to function, and how we might help make it function in that way. Cranial or jaw measurements could also help establish more nearly normal standards for comparison.

3. Electromyography: The use of this method allows an operator to analyse muscle activity by measuring and recording the amplitude and frequency of the electrical potentials within the muscle. Interest in this field was spurred by reports by Rogers about 20 or 30 years ago on the results achieved in treating malocclusion cases by myofunctional exercises. Most work in electromyography as it applied to the Orthodontics field has been done on the Temporalis muscle, because first of all it was close to the surface and its action potentials could be picked up by surface electrodes, and secondly the posterior position of the Temporal muscle is the retractor of the mandible and it was thought that overactivity of this portion may be a causative factor in certain Class II or Distocclusion cases. Moyers (about

1949 or 1950) reported hyperactivity in the posterior portion of Temporalis in some such cases.

4. Cephalometrics: The use of cephalometric radiograms provides another method of studying the dynamic changes that occur in the anatomy of the facial bones during growth and development of an individual. Measurements are made between anatomical points visible on the radiogram and comparisons are made between measurements of various groups of individuals or between measurements made on serial radiograms of the same individual. The X-ray pictures are made according to rigid standards, i.e. using a head-holder and standard tube angulation, exposure, etc.

One of the difficulties inherent in this type of work is that the inexperienced worker either ignores or tends to forget that the radiogram represents only shadows of the bones projected on the film. The tendency is to accept measurements of the shadows of bones, or parts of bones, without sufficient preliminary study of the influence of the morphology or rotation of the individual bones on the size of the shadow which is being studied. As an example, Dr. E. Harvold, the Norwegian dentist and anatomist who organized the cleft palate research and treatment centre at the Toronto Hospital for Sick Children some years ago, developed the technique for taking a certain oblique radiogram of the skull (the one presently recorded at Burlington, mentioned below) only after a long period of study using dried skulls to determine the head position and angulation that would give least distortion of the part to be studied.

One can, of course, tend to ignore these factors when large numbers of data are being processed in order to establish some basic relationship between structures, but interpretation of films is difficult in terms of individual patients who may differ from the group average in one or several aspects.

Cephalometrics has been of value in studying asymmetry of faces. Studies have been made to determine the average asymmetry in various groups of individuals. This is of importance in determining the relative amount of deformity in congenital defects.

Sources of Material:

The material which has been studied under Committee sponsorship has been derived chiefly from four sources:

1. Anthropological specimens belonging to various museums, as reported by Mitchell, Johns, and Culbert.
2. Patients being treated in the Orthodontic Clinic, Faculty of Dentistry, University of Toronto. The group under treatment

by Dr. Jarvis (Part II of Report of D.R. 37) was drawn from this source.

3. Some three or four years ago a group of 40 school children was assembled, each of whom had a classic "normal" dentition. It was intended to make serial studies of these children over the next several years to establish ranges of certain measurements in "normal" children of various ages, and to attempt to establish what went wrong if malocclusions developed in any of the group. This group has now been reduced in number to about 34, and the third study of them is presently being made.
4. Much work, both electromyographic and cephalometric, is presently being done on material collected at the Burlington Interceptive Orthodontic Centre. This centre was started in 1952 at Burlington, Ontario, with several purposes behind it:
 1. To evaluate a programme of interceptive orthodontics,
 2. To analyse and define the characteristics of normal occlusion,
 3. To analyse and define the characteristics of the various
 4. To determine the role of inheritance in determining a number of clinically significant characteristics of cranial and facial growth.

Records (including detailed case histories, casts of the dentition, and a set of radiographs including lateral, anteroposterior, and oblique angulations) were originally taken of some 1050 children, including 450 three-year-olds, and 150 each of 6, 8, 10, and 12 year olds. The latter four groups constituted the control. It was proposed to follow the original 450 three-year-old children for the following ten year period, taking serial records each year. Conservative treatment of any of this latter group who might exhibit signs of abnormality is being started early in order to evaluate the worth of such a procedure.

One cannot overemphasize the value of this particular study, especially as it involves the collection of data. The records being gathered are of the finest and it is anticipated that the final library of material will provide a source for researchers from all parts of the world for years to come.

Departmental Organization:

Prior to about 1949 dentists who wished to be certified as specialists in the field of Orthodontics (and others) were required to spend one academic post-graduate year in order to

satisfy the requirements of the licensing board. Beginning approximately seven years ago, the Department of Orthodontics in the Faculty of Dentistry, University of Toronto, switched to a two-year programme. Instead of receiving a Diploma (as formerly) for completion of their programme, students were required to register in the School of Graduate Studies and to complete the requirements for a Master of Science in Dentistry degree. The number of students taking the course was increased from a very few to six in each of the two years of the course. As part of the degree requirements, each candidate was obliged to submit a thesis reporting the results of an original research problem carried on by him in the Department. This, of course, greatly increased the number of research projects being carried on and nearly all of the projects being supported by this Committee date from this period.

The Department was headed from about 1949 to 1954 by a full-time Professor who held a Ph.D. degree in physiology from Iowa State University. He was responsible for developing interest in research, particularly in the fields of anthropology and electromyography.

For the last two years a new department head has been in charge. He was trained locally, and while he was a brilliant student, he had no basic science training other than that received while he attended the course given in the Department. He has been responsible for some excellent work in the field of cephalometrics, where his chief interest lay. He has been directing the Department on a half- or part-time basis, and although the number of post-graduate students has been reduced to four per year, still the volume of research required for the number of students to complete Master's degree requirements is considerable, and requires more than part-time supervision.

Beginning in the fall of 1956, some changes in Departmental organization are anticipated. For example, candidates who enrol for training leading to specialist certification in the field of Orthodontics (as in others) will not register in the School of Graduate Studies as candidates for the Master's degree. They will be registered in the Dental Faculty and will receive a diploma as recognition of successful completion of the course. This will relieve the load considerably as far as student research requirements are concerned, although it is anticipated that there will be certain departmental requirements in this field.

Summary and Conclusions:

1. Generally speaking, the results from projects being conducted by individuals on a part-time basis (the rest of the time being spent in practice) are not too satisfactory. Too

much of the worker's time and energy are devoted necessarily to the problems of practice for a good job to be done on the research.

2. It should be noted that while the men who take Orthodontic training in Toronto do get refresher courses in basic sciences, for example, gross anatomy, histology, physiology, etc., they should by no means be considered as trained in any of these fields. Indeed, that is not the objective of their training.

3. Results in the field of anthropological research are not yet known. This work has been conducted on a part-time basis by men who are trained as clinical orthodontists and not anthropologists or anatomists. Their sincerity in their work is overshadowed by the demands of practice.

4. In the field of electromyography the results have largely been negative. Data, carefully gathered over the last three or four years indicate that this cannot be expected to be a particularly fruitful field for research in the orthodontic field. To date no clear-cut positive correlations have been found between muscle activity and the occurrence of malocclusion. Those who have collected the data should be congratulated. It has been no easy job because of the tremendous technical difficulties in this type of work, and their results may be interpreted as quite positively negative.

5. Those projects which have to do with treatment (for example, DR 37, Part II) are proceeding very well, and final results are expected to be excellent ones.

6. Some results in cephalometric research have formed a sound contribution to knowledge in the field. Here, however, it is felt that with a man trained in research techniques and who recognizes the limitations of the methods directing research endeavours in the field, more profitable and less wasteful results might be expected. As the data presently being collected at the Burlington Centre continue to accrue, a veritable gold mine of material will be filed for use. With properly trained men directing and doing research with this material, we can have every right to expect great things from it.

Summary and Conclusions:

1. Generally speaking, the results from projects being conducted by individuals on a part-time basis (the rest of the time being spent in practice) are not too satisfactory. Top

HISTORY OF PROJECT DR-22

"An investigation of the mechanism of repair of the supporting structure of the teeth"

The original grant for this project was made in 1948-49 to Dr. H.K. Box, Research Professor of Periodontology at the University of Toronto. In October 1948, Dr. Box felt that his other work prevented him from exercising the necessary supervision of the project, and Dr. C.H. Best agreed to accept responsibility for directing the research. Applications have been received from Dr. Best, and awards made, annually since that time.

Since the initiation of the project in 1948, Dr. W.J. Linghorne, D.D.S., a Research Associate at the Banting and Best Dept. of Medical Research since 1948 and a Special Lecturer in the Faculty of Dentistry at the University of Toronto since 1952, has devoted half his time to the investigation and has been paid \$2,000 per annum under the grant.

In January, 1950, Dr. Best suggested to the Associate Committee on Dental Research that Dr. Linghorne be named grantee under this project. It was pointed out, however, that paragraph 3 of the general conditions governing the award of grants for research states specifically that:

"Grants are to be made only for the actual expenses of investigation. They are not to support the grantee or other members of the Institution staff in carrying out the investigation, but may be used in whole or in part for the payment of assistants..."

The change suggested by Dr. Best would therefore have meant that Dr. Linghorne could not be paid from the funds made available for the project, so Dr. Best agreed to continue to apply for the grant in his own name, and to employ Dr. Linghorne under the grant.

The question of a certain irregularity has arisen on numerous occasions, since it appears that the person paid under the grant (Dr. Linghorne) rather than the actual grantee (Dr. Best) is responsible for the research.

In reviewing the application for a grant for 1953-54, the Executive of the Associate Committee on Dental Research (11th Mtg. of the Exec., March 1953, Min. 2(i)) felt that the continued employment of any one scientist under a research grant

was detrimental to his own interest and that project DR-22 was sufficiently well established to permit withdrawal of the Committee's support at the end of the year 1953-54. The members of the Committee agreed that Dr. Linghorne should be regarded as a senior researcher in this field and that the Committee should not continue to support him as such indefinitely (17th Mtg., March 1953, Min. 28). The following letter was therefore sent to Dr. Best by the secretary on March 10, 1953:

"The Associate Committee on Dental Research has given careful consideration to the application for renewal of grant No. DR 22. The Committee shares the feeling of the U.K. Medical Research Council and many other similar bodies, that it is not desirable to make substantial contributions to the income of relatively senior scientific workers for more than a limited number of years, for the obvious reason that such workers may come to depend considerably on this income, whereas the granting body is by its terms of reference required to reconsider its commitments annually and has no certain continuance. The view was strongly expressed that this undesirable situation is arising in the case of Dr. Linghorne, especially since his researches appear to be expanding into new fields.

"There was a proposal that the request for renewal should be rejected; but the Committee, being decidedly interested in the re-attachment problem, and being reluctant to create an embarrassing situation for you and for Dr. Linghorne by withdrawing support at short notice, eventually agreed to accept this application for renewal of DR 22, but agreed also that Dr. Linghorne's partial personal dependence on these grants should not under any circumstances continue beyond the end of the year 1953-54."

Dr. Best was invited to attend the 18th Meeting of the Committee in October 1953 and he indicated (Min. 9) that he proposed to file application for a Senior Dental Research Fellowship for Dr. Linghorne to enable him to continue his study of the mechanism of repair during 1954-55. He was later discouraged in this by the Awards Officer, since Fellowships are awarded by Council only to those devoting full time to research, rather than the half time proposed in Dr. Linghorne's case. It was suggested that if Dr. Best

wished to continue paying Dr. Linghorne for his investigation, he would be more likely to receive the approval of Council by continuing to employ him under a grant. The resulting application to the Associate Committee on Dental Research for a grant for 1954-55 was duly approved by the Committee at its 19th Meeting (March 1954) and confirmed by Council.

The irregularity of the grant was again discussed at the 20th Meeting of the Committee in Oct. 1954 (Min. 5(iii)), and during the discussion it was pointed out that Dr. Linghorne, too, would undoubtedly be glad to have the situation regularized under some aspect of the Council's awards program. It was suggested that the irregularity could be overcome by having Dr. Linghorne's work supported under a consolidated grant, but the Director of the Division of Dental Research at the University of Toronto (Dr. Macdonald, Chairman of the Committee) said that no application for a consolidated grant to his Division was contemplated at that time.

No solution to the problem could be found, but in view of the value of Dr. Linghorne's work to the dental profession, it was resolved

"THAT the Committee continue to receive annually applications from Dr. Best for grants in support of the work of Dr. Linghorne in the hope that a satisfactory and permanent solution to the problem of continuing financial support will be effected by appropriate action on the part of the Associate Committee on Dental Research in concert with other parties concerned."

DR-4	Completed	Brig. E.M. Wandbrough, Royal Can. Dental Corps.	To study the effects of altitude acclimatization and dental adaptation on oral hygiene.
DR-5	Completed	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University.	Critical survey of the literature on dental caries.
DR-6	Completed	Dr. A.D.A. Mason, Faculty of Dentistry, Univ. of Toronto.	A study of the dental caries of the children of hospital wards in the institution of Ontario for the blind.

APPENDIX T

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

List of Grantees and Projects

<u>Project No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-1	Completed	Dr. J.J. Rae, Dept. of Chemistry, Univ. of Toronto.	Chemical mechanisms in dental caries.
DR-2	Completed	Dr. G.H.W. Lucas, Dept. of Pharmacology, Univ. of Toronto.	A study of alveolar oxygen tension and blood oxygen con- tent during nitrous oxide anaesthesia.
DR-3	Completed	Dr. H.K. Box, Faculty of Dentistry, Univ. of Toronto.	A study of micro- organisms found in deep periodontal pockets and caries lesion as revealed by the electro- microscope.
DR-4	Completed	Dr. H.K. Box, Faculty of Dentistry, Univ. of Toronto.	An investigation of packing material in the treatment of periodontal disease.
DR-5	Completed	Major R.L. Twible, Royal Cdn. Dental Corps (later O.R.F.)	To improve certain properties of the acrylic resin den- ture base and to effect an economy in denture produc- tion.
DR-6	Completed	Brig. E.M. Wansbrough, Royal Cdn. Dental Corps.	To study the effects of altitutde acceler- ation and deceler- ation on oral tissues.
DR-7	Completed	Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University.	Critical survey of the literature on dental caries.
DR-8	Completed	Dr. A.D.A. Mason, Faculty of Dentistry, Univ. of Toronto.	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply.

<u>Grant No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-9	Completed Dr. O.J. Walker Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, Univ. of Alberta.	An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry.
DR-10	Completed Dr. G.M. Macdonald Queen Mary Veterans Hospital, Montreal.	Facial prostheses.
DR-11	Completed Dr. R.J. Godfrey, Faculty of Dentistry, Univ. of Toronto.	An anatomical, histological and physiological study of the role of the condyle in mastication.
DR-12	Completed Dr. A.E.R. Westman, Ont. Res. Foundation.	Investigation of cements for methacrylate inlays.
DR-13	Completed Dr. M. Doreen Smith, Dept. of Food Chemistry, Univ. of Toronto.	Fluoride content of dentine, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford.
DR-14	Completed Dr. M. Doreen Smith, Dept. of Food Chemistry, Univ. of Toronto.	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries.
DR-15	Completed Dr. N. F. Walker, Dept. of Zoology, Univ. of Toronto.	A comparison of the patterns of eruption of the deciduous teeth in man with rate of growth of the dental arches.
DR-16	Completed Dr. C.H. Best, Dept. of Medical Res., Univ. of Toronto.	Studies in vitro of certain fungus-like organisms found in periodontal diseases.
DR-17	Completed Dr. R. F. Shaner, Dept. of Anatomy, Univ. of Alberta.	An investigation of effects of solutions used in operative dentistry on the vital pulps of teeth.

<u>Project No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-18	Completed	Dr. H.K. Box Faculty of Dentistry, Univ. of Toronto.	Experimental production of dental caries.
DR-19	Completed	Dr. E.A. Sellers, Dept. of Pharmacology, Univ. of Toronto.	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry.
DR-20	Completed	Dr. Jules Thebaud, Faculty of Dental Surgery, Univ. of Montreal,	Improvement of subgingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris.
DR-21	Completed	Dr. A.E.R. Westman, Ont. Res. Foundation.	Investigation of repair techniques for methacrylate dentures.
DR-22	Active	Dr. C.H. Best, Dept. of Medical Res., Univ. of Toronto.	Investigation of the mechanism of repair of the supporting structures of the teeth.
DR-23	Active	Dr. C.P. Leblond, Dept. of Anatomy, McGill University.	Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.
DR-24	Completed	Dr. C.H.M. Williams, Faculty of Dentistry, Univ. of Toronto.	The effects of hard and soft diets on the supporting structures of the teeth.
DR-25	Completed	Dr. A.E.R. Westman, Ont. Res. Foundation.	Investigation of methods for the use of methyl methacrylate in direct dental fillings.
DR-26	Completed	Dr. R.E. Moyers, Faculty of Dentistry, Univ. of Toronto.	A study of the normal and abnormal physiologic forces of the oral cavity.
DR-27	Completed	Dr. J.W. Abbiss, Faculty of Dentistry, Dr. A.G. Nutlay, Faculty of Dentistry, Dalhousie University.	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth.

<u>Project No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-28	Completed	Dr. O.J. Walker Dept. of Chemistry, Univ. of Alberta.	Investigation of methods for destroying the organic matter in teeth, bones and other materials prior to determination of fluorine content.
DR-29	Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, Univ. of Toronto.	Biological absorption of fluorine.
DR-30	Completed	Dr. N.F. Walker, Dept. of Zoology, Univ. of Toronto.	The study of the closure of space following premature loss of deciduous teeth.
DR-31	Completed	Dr. L.B. Jaques, Dept. of Physiology, Dr. G.J. Millar, Dept. of Physiology, Univ. of Saskatchewan.	An investigation of nerve block.
DR-32	Completed	Dr. A. W. Ham, Dept. of Anatomy, Univ. of Toronto.	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structure of the growing incisor teeth of animals.
DR-33	Completed	Dr. H.A. Hunter, Faculty of Dentistry, Univ. of Toronto.	A study of calcifying potential of calcium oleate on the dental pulp.
DR-34	Completed	Dr. A. D'Iorio, Dept. of Physiology, Univ. of Montreal.	Studies of calcium metabolism in teeth with the aid of Ca^{45} .
DR-35	Completed	Dr. J.B. Macdonald, Div. of Dental Res., Univ. of Toronto.	Bacteriology of periodontal disease. I. Experimental fusospirochetal infection with mixtures of pure culture.
DR-36	Completed	Dr. G. Nikiforuk, Div. of Dental Res., Univ. of Toronto.	Demineralization of hard tissues as bone and teeth by organic chelating agents.

<u>Project No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-37	Completed	Dr. D. H. Jenkins, Dept. of Orthodontics, Univ. of Toronto.	Further studies in the electromyography of the head, face and neck.
DR-38	Completed	Dr. K.J. Paynter, Faculty of Dentistry, Univ. of Toronto.	The effects of the endocrines on the teeth and jaws. II. Further studies on the effect of thiouracil on the development of molar teeth of rats.
DR-39	Completed	Dr. A.G. Racey, Faculty of Dentistry, McGill University.	Sectioning of teeth and surrounding struc- tures and the effects of potassium deficiency and of pyridoxine de- ficiency on the teeth and surrounding struc- tures of dogs.
DR-40	Completed	Dr. L.A. Funt, Faculty of Dentistry, Univ. of Toronto.	Effect of tooth erup- tion and function on the growing of the mandible and facial complex on cats.
DR-41	Completed	Dr. D. H. Jenkins, Dept. of Orthodontics, Univ. of Toronto.	A study of the dental arch form and certain of its anatomical re- lations in a series of clinically excellent dentitions.
DR-42	Completed	Dr. G. Nikiforuk, Div. of Dental Research, Univ. of Toronto.	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators.
DR-43	Completed	Dr. S.G. Davis, Dept. of Chemistry, Dr. H.R. MacLean, Faculty of Dentistry, Univ. of Alberta.	Electro-potentials caused by dental alloys.

<u>Project No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-44	Completed	Dr. A.H. Craven, Faculty of Dentistry, McGill University.	Facial and cranial growth patterns in the rat as revealed by vital staining with alizarine red S.
DR-45	Completed	Dr. R.E. Moyers, Faculty of Dentistry, Univ. of Toronto.	Study of the incidence of malocclusion.
DR-46	Completed	Dr. D. H. Jenkins, Dept. of Orthodontics, Univ. of Toronto.	Study of treated cases.
DR-47	Completed	Dr. H.A. Hunter, Faculty of Dentistry, Univ. of Toronto.	The pathological characteristics of experimental fusospirochetal infections.
DR-48	Completed	Dr. G. Nikiforuk, Div. of Dental Research, Univ. of Toronto.	The effect of topical application of silicons in protecting teeth against acid solutions in vitro and dental caries produced in vitro.
DR-49	Completed	Dr. A. H. Craven, Faculty of Dentistry, Univ. of Toronto.	A recording device to determine postural changes of the head in the horizontal plane.
DR-50	Completed	Dr. J.B. Macdonald, Div. of Dental Research, Univ. of Toronto.	The cultivation and characteristics of <u>Bacteroides nigrescens</u> .
DR-51	Active	Dr. R.L. deC. H. Saunders Dept. of Anatomy, Dalhousie University.	Study of human dental pulp blood vessels by X-ray microarteriographic methods.
DR-52	Active	Dr. L.F. Belanger, Dept. of Histology & Immunology, Univ. of Ottawa.	Mechanism of bone and tooth formation in the light of autoradiography.
DR-53	Completed	Dr. C.B. Weld, Dept. of Physiology, Dalhousie University.	The macrophages in the pulp tissue of the rat incisor and lower jaw.

<u>Project No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-54	Completed	Dr. B.N. Kropp, Faculty of Medicine, Queen's University.	Development of a rapid method for grinding thin sections of plastic-embedded teeth.
DR-55	Completed	Dr. M.R. Culbert, Dept. of Orthodontics, Univ. of Toronto.	A cephalometric study of maxillary growth as related to statural growth.
DR-56	Completed	Dr. D.J.E. Mitchell, Dept. of Orthodontics, Univ. of Toronto.	Comparative study of palatal height, width and length in ancient and modern crania.
DR-57	Active	Dr. G. Nikiforuk, Div. of Dental Research, Univ. of Toronto.	The amino acid composition of enamel protein using paper chromatography.
DR-58	Active	Dr. J.G. Aldous, Dept. of Pharmacology, Dalhousie University.	The metabolism of procaine and related compounds.
DR-59	Active	Dr. D. H. Copp, Dept. of Physiology, Dalhousie University.	Effect of phosphorus deficiency on bones and teeth.
DR-60	Active	Dr. F.C. Fraser Dept. of Genetics, McGill University.	Pathogenesis of cortisone-induced cleft palate in mice.
DR-61	Active	Dr. A.S.V. Burgen, Dept. of Physiology, McGill University.	Secretion of protein in saliva.
DR-62	Completed	Dr. S. Kirkwood, Dept. of Biochemistry, McMaster University.	Role of the salivary glands in the extra-thyroidal metabolism of the thyroid hormone.
DR-63	Active	Dr. K.J. Paynter, Div. of Dental Res., Univ. of Toronto.	A histological study of teeth and periodontal tissues of guinea pigs reared on subminimal allowances of ascorbic acid.
DR-64	Active	Dr. K.J. Paynter, Div. of Dental Res., Univ. of Toronto.	Investigations into the nature of cementum.

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-65	Active Dr. G. Pelletier, Dr. J.G. Perreault, Faculty of Dentistry, Univ. of Montreal.	An evaluation of the disturbance of the dentine formation in the rat incisor following trauma of various origins.
DR-66	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-67	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-68	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Study of the dental helix, which is present in ancient and modern crania.
DR-69	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-70	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-71	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-72	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-73	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-74	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-75	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-76	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-77	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-78	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-79	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-80	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-81	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-82	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.
DR-83	Active Dr. G. Perreault, Faculty of Dentistry, Univ. of Montreal	Study of the histology of the dentine and its relation to the pulp.
DR-84	Completed Dr. D.B. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of the dental helix, which is present in ancient and modern crania.

APPENDIX U

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Papers Published

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
1	New aspects of periodontal re- search, by H.K. BOX.	Presented at National Convention of Can. Den- tists, Toronto, May 29, 1946 (App. C, 5th Mtg., A.C.D.R., 25 Feb. 1947)
2	Concerning the pathogenesis of periodontal disease, by H.K. BOX.	J. Periodontology 19: 11-20, 1948.
3	The effect of various inorganic salts on the solubility of cal- cium phosphate, tooth enamel and whole teeth in lactic acid, by J.J. RAE and C.T. CLEGG.	J. Dent. Res. 27: 52-53, 1948.
4	Changes in the calcium and phos- phate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J.J. RAE and C.T. CLEGG.	J. Dent. Res. 27: 54- 57, 1948.
5	An improved method for process- ing acrylic dentures, by D.R. CHRISTIE.	J. Can. Dent. Assoc. 14: 303-317, 1948.
6	The therapeutic properties of periodontal cement packs, by LINGHORNE and D.C. O'CONNELL.	J. Can. Dent. Assoc. 15: 199-205, 1949.
7	Phosphatase in saliva, by J.T. DENTAY and J.J. RAE.	J. Dent. Res. 28: 68- 71, 1949.
8	The treatment of acute oral Vincent's infection, by W.J. LINGHORNE and D.F. STEDMAN.	J. Can. Dent. Assoc. July 1949.
9	A comparison of nine local an- aesthetics, by H.S. HAMILTON, B.A. WESTFALL and J.K.W. FERGUSON	J. Pharmacol. Expt. Ther. 94: 299-307, 1948.
10	New methods of repairing acrylic dentures without distortion, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 15: 582-590, 1949.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D.E.R. COGGLES, O.J. WALKER and H.R. MacLEAN.	J. Can. Dent. Assoc. 16: 75-82, 1950.
12	The relation between buffering capacity, viscosity and lactobacillus count of saliva, by J.J. RAE and C.T. CLEGG.	J. Dent. Res. 28: 589-593, 1949.
13	Mineralization of the growing tooth as shown by radiophosphorus autographs (17692), by L.F. BELANGER and C.P. LEBLOND.	Proc. Soc. Expt. Biol. Med. 73: 390-391, 1950.
14	Radio-autographic visualization of bone formation in the rat, by C.P. LEBLOND, G.W. WILKINSON, L.F. BELANGER and J. ROBICHON.	Am. J. Anat. 86: 2, 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH.	Can. J. Research, F, 28: 227-233, 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W.J. LINGHORNE and D.C. O'CONNELL.	J. Dent. Res. 29: 419-428, 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH.	J. Can. Dent. Assoc. Jan., 1951.
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J.P. LUSSIER.	Rev. Canadienne de Biol. 10: 175-180, 1951.
19	Relining acrylic dentures without distortion, by D.R. CHRISTIE	J. Can. Dent. Assoc., July, 1951.
20	Acrylic fillings - general comments and some experimental data, by D.R. CHRISTIE.	J. Can. Dent. Assoc., 17: 427-435, 1951.
21	Ammonia production in saliva, by R.M. BALLANTYNE, C.T. CLEGG, J.J. RAE and F.H. LAWFORD.	J. Dent. Res. 30: 30: 385-387, 1951.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
22	Studies in the regeneration and reattachment of supporting structures of the teeth. II. Regeneration of alveolar process, by W.J. LINGHORNE and D.C. O'CONNELL.	J. Dent. Res. 30: 604-614, 1951.
23	Radioactive isotopes in dental research, by J.P. LUSSIER and A.D'IORIO.	The Brit. Dent. Annual, 1952.
24	Determination of fluoride in water by the aluminum-hematoxylin method, by MARGARET J. PRICE and O.J. WALKER.	Anal Chem. 24: 1593, 1952.
25	Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat, by L.F. BELANGER.	Science 118: 520-521, 1954.
26	The motile non-sporulating anaerobic rods of the oral cavity, by J.B. MACDONALD.	University of Toronto Press, Toronto, 1953.
27	Motility in a species of non-flagellated bacteria (20677), by J.B. MACDONALD, M.L. KNOLL and R.M. SUTTON.	Proc. Soc. Expt. Biol. & Med. 84: 459-462, 1953.
28	The periodontium and salivary glands in the alarm reaction, by G. SHKLAR and I. GLICKMAN.	J. Dent. Res. 32: 773-778, 1953.
29	Autoradiographic visualization of S^{35} incorporation and turnover by the mucous glands of the gastro-intestinal tract and other soft tissues of rat and hamster, by L.F. BELANGER.	Anat. Rec. 118: 755-772, 1954.
30	Autoradiographic visualization of the entry and transit of S^{35} in cartilage, bone, and dentine of young rats and the effect of hyaluronidase in vitro, by L.F. BELANGER.	Can. J. Biochem. & Physiol. 32: 161-169, 1954.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
31	Autoradiographic visualization with Ca^{45} of normal growth of the incisor of pigs and the effect of fluorine feeding, by L.F. BELANGER, W.E. LOTZ, W.J. VISEK and C.L. COMAR.	Anat. Rec. 119: 53-70, 1954.
32	Fluorine balance studies of four infants, by MARY P. HAM and M. DOREEN SMITH.	J. Nutrition 53: 215-223, 1954.
33	Fluorine balance studies of three women, by MARY P. HAM and M. DOREEN SMITH.	J. Nutrition 53: 225-232, 1954.
34	The effect of propylthiouracil on the development of molar teeth, by K.J. PAYNTER.	J. Dent. Res. 33: 364-376, 1954.
35	Autoradiographic detection of radiosulfate incorporation by the growing enamel of rats and hamsters, by L.F. BELANGER.	J. Dent. Res. 34: 20-27, 1955.
36	Autoradiographic visualization of Ca^{45} intake by normal and pathological cartilage in vitro, by L.F. BELANGER.	Proc. Soc. Expt. Biol. & Med. 88: 150-152, 1955.
37	Studies in the reattachment and regeneration of the supporting structures of the teeth. III. Regeneration in epithelized pockets, by W.J. LINGHORNE and D.C. O'CONNELL.	J. Dent. Res. 34: 164-177, 1955.

APPENDIX V

Members of the Associate Committee on Dental Research & its Executive, with their terms of appointment

	<u>Term to expire</u>
★ 1. Dr. J.B. Macdonald, <u>Chairman</u> , Division of Dental Research, University of Toronto, 230 College St., Toronto 2-B, Ontario.	31 March, 1957
2. Dr. E. W. R. Steacie, <u>ex officio</u> President, National Research Council, Ottawa 2, Canada.	- -
3. Dr. E.G. D. Murray, <u>ex officio</u> , Dept. of Medical Research, University of Western Ontario, London, Ontario.	- -

Representing the Dental Profession

4. Dr. J.S. Bagnall, Faculty of Dentistry, Dalhousie University, Halifax, N.S.	31 March, 1958
5. Dr. C.D. Husband, Suite 110, Metro Block, Red Deer, Alberta.	31 March, 1956
6. Dr. Jean-Paul Lussier, Dept. of Dental Surgery, University of Montreal, Montreal, P.Q.	31 March, 1957
★ 7. Dr. A. G. Racey, Faculty of Dentistry, McGill University, Montreal, P.Q.	31 March, 1956
8. Dr. C.H.M. Williams, Faculty of Dentistry, University of Toronto, Toronto 5, Ontario.	31 March, 1957

★ Member of Executive

Term to expire

Representing the Royal Canadian Dental Corps,
Dept. of National Defence

- ★ 9. Brig. E.M. Wansbrough, ex officio, - -
Director General of Dental Services,
Royal Canadian Dental Corps,
Dept. of National Defence,
Ottawa, Ontario.

Representing the Division of Dental Health,
Dept. of National Health and Welfare,

10. Dr. H.K. Brown, ex officio, - -
Chief, Dental Health Division,
Dept. of National Health and Welfare,
Ottawa, Ontario.

Representing the Dental Research Div.,
Dept. of Veterans Affairs.

11. Dr. L.A. Kilburn, ex officio, - -
Director of Dental Research,
Dept. of Veterans Affairs,
Ottawa, Ontario.

Representing the Basic and Medical Sciences

12. Dr. J.G. Aldous, 31 March, 1956
Dept. of Pharmacology,
Dalhousie University,
Halifax, N.S.
13. Dr. H.J. Barrie, 31 March, 1957
Dept. of Pathology,
Faculty of Medicine,
University of Toronto,
Toronto, Ontario.
14. Dr. J.M.R. Beveridge, 31 March, 1956
Dept. of Biochemistry,
Queen's University,
Kingston, Ontario.
15. Dr. A. C. Burton, 31 March, 1956
Dept. of Biophysics,
University of Western Ontario,
London, Ontario.
16. Dr. D. H. Copp, 31 March, 1956
Dept. of Physiology,
University of British Columbia,
Vancouver 9, B.C.

★ Member of Executive

INITIAL DISTRIBUTIONTerm to expire

- ★ 17. Dr. A. W. Ham,
Dept. of Anatomy,
University of Toronto,
Toronto, Ontario. 31 March, 1957
18. Dr. Edouard Pagé,
Director, Institute of Biology,
University of Montreal,
Montreal, P.Q. 31 March, 1958
- ★ 19. Dr. J. B. Marshall, Secretary,
Awards Officer,
National Research Council,
Ottawa 2, Ontario. - -

★ Member of Executive.

- (11) To other persons:-
- 20-25 Deans of Dental Schools (who are not members of the
Committee)
20. Alberta - Dr. W.B. Hamilton
21. Dalhousie - Dr. J.B. McLean
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Associate Committee on Dental Research

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| 2. Dr. J.G. Aldous | 12. Dr. J-P Lussier |
| 3. Dr. J.S. Bagnall | 13. Dr. E.G. Murray |
| 4. Dr. H.J. Barrie | 14. Dr. E. Pagé |
| 5. Dr. J.M.R. Beveridge | 15. Dr. A.G. Racey |
| 6. Dr. H.K. Brown | 16. Dr. E.W.R. Steacie |
| 7. Dr. A.C. Burton | 17. Brig. E.M. Wansbrough |
| 8. Dr. D.H. Copp | 18. Dr. C.H.M. Williams |
| 9. Dr. A.W. Ham | 19. Dr. J.B. Marshall,
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| 10. Dr. C.D. Husband | |

(ii) To other persons:-

20-23 Deans of Dental Schools (who are not members of the Committee)

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
FIFTEENTH MEETING
OF THE
EXECUTIVE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

4 MARCH 1957

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Fifteenth Meeting

of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Council Chamber, National Research Council, at 4:15 p.m., March 4th, 1957.

1. Attendance

Dr. J. B. Macdonald, Chairman
Dr. D. H. Copp
Dr. A. G. Racey
Brig. E. M. Wansbrough
Dr. J. B. Marshall, Secretary
Miss D. J. Wright

Apologies had been received from Dr. Ham, who had sent his comments and recommendations regarding grant applications.

2. Applications for Grants

Careful consideration was given to 11 applications for grants in aid of research totalling \$38,361. The following recommendations were made for presentation at the second session of the Committee on March 5th.

Aldous, J. G.
Dept. of Pharmacology
Dalhousie University

The metabolism of procaine hydrochloride and related compounds. IV and V.

(DR-58)

APPROVED \$3,100.

Burgen, A. S. V.
Dept. of Physiology
McGill University

Synthesis and secretion of protein in saliva.

(DR-61)

APPROVED \$5,930.

Belanger, L. F.
Dept. of Histology & Embryology
University of Ottawa

The intimate mechanism of mineralization.

(DR-52)

APPROVED \$3,500.

- Copp, D. H.
Dept. of Physiology
University of British
Columbia
Phosphorus deficiency:
Effects on bones and
teeth.
(DR-59) APPROVED \$6,284.
- Leblond, C. P.
Dept. of Anatomy
McGill University
Entry of carbohydrates
into teeth as shown
with the help of radio-
active elements.
(DR-23) APPROVED \$3,500.
- Linghorne, W. J.
Banting Institute
University of Toronto
Investigation of the
mechanism concerned in
the maintenance and
repair of the suppor-
ting structures of the
teeth.
(DR-22) NOT APPROVED
- Melville, K. I.
Dept. of Pharmacology
McGill University
Studies on the mechanism
of gingival changes
associated with drug
administration.
(DR-66) APPROVED \$4,300.
- Paynter, K. J.
Div. Dental Research
University of Toronto
Investigation into the
nature of cementum.
(DR-64) APPROVED \$3,400.
- Perreault, G.
Faculty of Dentistry
University of Montreal
Effects of acute and
chronic trauma on the
pulp and growing den-
tin in the incisors
of albino rats.
(DR-65) APPROVED \$2,595.
- MacLean, H. R.
Faculty of Dentistry
University of Alberta
The role of epithelium
in the genesis of
anomalies of tooth
development.
ENCUMBER \$2,252.
and ask applicant
for further infor-
mation as to what
he proposes to do.
- McMurchy, K. A.
Faculty of Dentistry
University of Alberta
A study of resorption
of enamel, dentin and
cementum.
ENCUMBER \$200.
and ask applicant
for further infor-
mation as to his
proposed study.

3. Non-recurring Grant for Equipment

The Executive agreed that it should recommend to the Committee the award of a non-recurring grant of \$5,000 to Dr. Saunders to enable him to purchase the X-ray microscope for which he had applied, since it was doubtful that Council would have sufficient funds available during the next year to make the award it had already approved in principle.

4. Application for Fellowship

In view of the information obtained from Dr. Burgen, it was agreed that Dr. Weiss' application for a renewal of his Fellowship be not approved. It was agreed, however, that he might be employed under Dr. Burgen's grant if this were agreeable to Dr. Burgen, and additional funds could be provided to Dr. Burgen for this purpose if necessary.

5. Travel Grants

It was agreed that the applications from Dr. K. J. Paynter, Dr. G. Nikiforuk and Dr. A. M. Hunt for travel grants to enable them to attend the meeting of the International Association for Dental Research be recommended to the Committee for approval.

6. Summer Research Studentships

Dr. Copp outlined a proposal discussed with him by Dr. Lussier that summer research grants be made to dental students to enable them to assist on research projects being carried out in universities. Dr. Marshall advised the Executive that grants could not be made, but suggested that undergraduate students might be employed in research laboratories, as they are in the Council's laboratories, during the summer months. It was agreed that this would be an excellent way of giving students research training, but it was pointed out that to be of interest to the students involved, the research should have some bearing on dentistry. It was suggested that such employment might be offered in Dalhousie University and the Universities of Toronto, Montreal, Ottawa and British Columbia where research of high quality is going on. It was not considered important that the research actually was being done outside the dental faculty as long as it had some dental slant. It was agreed that Dr. Lussier should be asked to outline his proposal to the Committee at the second session.

7. Membership of the Committee

The Chairman reported that the terms of office of Dr. Lussier, Dr. Williams, Dr. Barrie, himself, Dr. Ham and Dr. Burton would expire in 1957; he also reported that Dr. Bagnall wished to resign from the Committee and if his resignation was accepted by the Committee, he too would have to be replaced. Various names were suggested, to be presented to the Committee for its consideration, together with nominations which might come from the members.

The Executive also agreed to recommend to the Committee the enlargement of the Executive by one member. It was pointed out that Dr. Ham would no longer be on the Executive during the coming year because he had served two terms on the Committee and was therefore ineligible for re-appointment at the present time. It was agreed to recommend to the Committee that Dr. Beveridge and Dr. Lussier be named to the Executive, along with Dr. Copp, Dr. Racey and Brig. Wansbrough.

8. Next Annual Meeting

The question of whether it would be wise to hold the next annual meeting at a university at which there was a dental school, e.g. Montreal, was discussed. It was suggested that if this were done, some open sessions might be held, and a guest speaker invited to give a lecture. It was thought that in this way, further stimulus might be given to dental research in Canada. Further discussion was held, but no definite decision was made.

The meeting adjourned at 6:45 p.m.

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Associate Committee on Dental Research

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| 8. Dr. D. H. Copp | 18. Brig. E. M. Wansbrough |
| 9. Dr. A. W. Ham | 19. Dr. C. H. M. Williams |
| 10. Dr. C. D. Husband | 20. Dr. J. B. Marshall, <u>Secretary</u> |

(ii) To other persons:-

21 - 24 Deans of Dental Schools (who are not members of the Committee)

- | | | |
|---------------|---|-----------------------|
| 21. Alberta | - | Dr. W. S. Hamilton |
| 22. Dalhousie | - | Dr. J. D. MacLean |
| 23. McGill | - | Dr. D. Prescott Mowry |
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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-THIRD MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

MARCH 4 - 5, 1957

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- Appendix P Cultural requirements of Bacteroides nigrescens.
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- Appendix R List of Publications.
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Initial Distribution.

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Dr. A. G. Hacey
Dr. C. H. M. Williams
Brig. E. M. Wetherburgh

Dr. J. H. Marshall, Secretary
Miss D. J. Wright

By Invitation

Dr. L. F. Belanger
Dr. A. S. V. Burgen

Apologies for their absence were received from

Dr. J. S. Bagnall
Dr. A. C. Burton
Dr. A. W. Ham
Dr. C. H. Husband
Dr. S. Page

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Twenty-third Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Held in the Council Chamber, National Research Council,

March 4 - 5, 1957

The twenty-third meeting of the Associate Committee on Dental Research was convened at 9:30 a.m., March 4, 1957.

1. Attendance

Dr. J. B. Macdonald, Chairman

Dr. J. G. Aldous

Dr. H. J. Barrie

Dr. J. M. R. Beveridge

Dr. H. K. Brown

Dr. D. H. Copp

Dr. L. A. Kilburn

Dr. J. P. Lussier

Dr. R. J. O'Neil

Dr. A. G. Racey

Dr. C. H. M. Williams

Brig. E. M. Wansbrough

Dr. J. B. Marshall, Secretary

Miss D. J. Wright

By invitation

Dr. L. F. Belanger

Dr. A. S. V. Burgen

Apologies for their absence were received from

Dr. J. S. Bagnall

Dr. A. C. Burton

Dr. A. W. Ham

Dr. C. H. Husband

Dr. E. Pagé

2. Chairman's Remarks

The CHAIRMAN opened the meeting by welcoming the new member of the Committee, Dr. R. J. O'Neil of Vancouver, and the guests, Dr. L. F. Belanger of the University of Ottawa and Dr. A. S. V. Burgen of McGill University.

The CHAIRMAN reported that at its 196th meeting, Council had appointed Dr. D. H. Copp to succeed him as Chairman of the Associate Committee on Dental Research. He felt that the Council was to be congratulated on its choice and said that the Committee was fortunate in having such a widely respected scientist playing a leading role in its activities.

During the course of his ensuing remarks, the CHAIRMAN referred to the rosy future for Canada's economy predicted by the Gordon Report and said that if the recommendations of the Commission are acted upon, research in the universities would undoubtedly be strengthened. He pointed out again the great need to train people, particularly dentists, in dental research; of recent years most of the projects sponsored by the Committee have been carried out by investigators in the basic science departments of Canadian universities, since research activity in the majority of the dental schools had really not yet begun. This trend had done much to raise the quality of fundamental research in problems relating to dentistry, but the small number of applications for grants from dental faculties was a matter of some concern. The Committee had tried over the past few years to arouse interest in research in the dental faculties by making fellowships available to dental graduates, but the stipends offered had apparently been too low to be attractive. The CHAIRMAN also felt that due to the apathy towards research apparent in most dental faculties, undergraduate students could see little opportunity for a rewarding career in research in Canadian dental schools. The Canadian Dental Association has tried to interest the deans of dental schools in stimulating research by offering a research scholarship to one student from each dental school, on the condition that a position on a dental faculty be assured following tenure of the scholarship; so far the response to this scheme has been discouraging.

The CHAIRMAN pointed out that dental disease in Canada is a 75 million dollar problem and a more realistic attitude has to be adopted in order to combat it. He felt that in the face of this problem it should not be too much to expect that each dental school should have one career research man on its staff at a salary as high as \$10,000. He expressed the opinion that such a development would be more effective

in stimulating interest in research and in training potential research investigators than what is now being done by means of small grants to established investigators in basic science departments. He added that the Council had established the type of appointment he had in mind when it instituted the Medical Research Associateship programme, and felt that a similar plan of Dental Research Associateships might be inaugurated in order to give impetus to dental research. It was acknowledged that at the present time such appointments cannot be made because there are as yet no suitable candidates in Canada for them, but it was felt that if such appointments had the expressed approval of agencies such as the Council, and the active support of the dental schools, dental graduates might be persuaded that opportunities for a career in research did in fact exist.

Meanwhile, the CHAIRMAN felt that for the next few years the main objective of the Committee should be not only the support of projects but also promotional work to encourage the development of fundamental research in dentistry.

3. Minutes of the Twenty-second Meeting

It was AGREED that the minutes of the Twenty-second Meeting be taken as read and approved.

4. Business Arising from the Minutes

(i) Fluoride analysis of teeth (22nd Mtg., Min. 4(i))

Dr. BROWN reported that he had a good supply of teeth - 121 permanent and 222 deciduous - on hand from the Brantford study, and he would like to see work on fluoride analysis continued. The technical difficulty was recognized, but the CHAIRMAN read to the meeting a reply Dr. COPP had received to his letter of inquiry to Dr. H. C. Hodge, Professor of Pharmacology and Toxicology of the University of Rochester. Dr. Hodge would be glad to have someone visit his laboratory for a week or two to learn the technique used there and it was agreed that advantage should be taken of this offer if the work is to be continued.

Dr. BROWN said too that he was strongly in favour of using the teeth for other purposes as well, such as histological studies of the structure of teeth. It was

suggested that qualified investigators such as Dr. Leblond and Dr. Nikiforuk might be interested in having the teeth for their experiments. Brig. WANSBROUGH asked whether funds might be available from the Brantford study, which is being carried out under the auspices of the Department of National Health and Welfare, to carry on basic research on the teeth available. It was pointed out that survey work could be supported by the Department of National Health and Welfare but that other aspects would more properly fall within the scope of the Associate Committee on Dental Research.

The Committee felt that it should not approach laboratory investigators with a project and it was therefore AGREED that Dr. BROWN might advise competent investigators of the availability of the material from the Brantford study in case they might wish to make use of it.

(ii) Action on Dr. Melville's application (22nd Mtg., Min. 9(ii))

The CHAIRMAN reported that additional information had been obtained regarding Dr. Melville's proposed investigation and, after a letter ballot of the Executive, a grant of \$3,000 had been made to him for 1956 - 57.

(iii) Fellowship stipend (22nd Mtg., Min. 13)

The CHAIRMAN reported to the meeting that a submission had been forwarded to Council recommending that the stipend for the Graduate Dental Research Fellowships be raised from \$2,000 - 3,500 to \$3,000 - 4,500. The decision of Council was read and it was noted that the present range approved by Council is \$2,200 - 4,000.

(iv) Inquiries re a Canadian Journal of Dental Research (22nd Mtg., Min. 18)

The CHAIRMAN reported that since the last meeting of the Committee he had reviewed the amount of work going on in Canada which might be published in a Canadian Journal of Dental Research if such a periodical were established. He was of the opinion that the maximum number of papers at the present time would be from 25 to 30 per year. Since it was felt that a minimum of 40 to 50 worthwhile papers would have to be submitted each year to justify the bimonthly publication of a dental journal, it was AGREED that no further action should be taken at this time to establish a new journal. It

was suggested that the matter might again be considered when the proposed new dental schools have been established.

Dr. COPP suggested that in the meantime it might be feasible to have a special section of the Journal of the Canadian Dental Association devoted to the publication of scientific papers. He felt that this would have the dual advantage of interesting dentists in research, and giving investigators an opportunity for more speedy publication of their papers. It was pointed out that certain standards of quality would have to be established, and this would involve the appointment of a scientific editor and the refereeing by appropriate scientific men of papers submitted. Following further discussion, it was MOVED by Dr. Copp and SECONDED by Dr. Williams that the following recommendation be forwarded to the Canadian Dental Association for consideration at the next meeting of its Board of Governors:

"The Associate Committee on Dental Research of the National Research Council of Canada, at a meeting held in Ottawa on March 4th and 5th, 1957 proposed the following recommendation:

"That the Canadian Dental Association consider the possibility of making available a section in the Journal of the Canadian Dental Association for publications on dental research originating in Canada; and further that a suitable editorial committee be set up to review papers submitted for publication in this section."

5. Consideration of Reports from Grantees

Reports from visiting grantees, Dr. Belanger and Dr. Burgen, were received first, but for ease of reference, reports are listed here in numerical sequence by project number.

(1) DR-22: Dr. C. H. Best (Dr. W. J. Linghorne)
Reported by Dr. Barrie

"An investigation of the mechanism of repair of the supporting structures of the teeth."

A summary and progress report appear in APPENDIX G.

Dr. BARRIE advised the Committee that the report submitted by Dr. Linghorne was essentially the substance of two seminars that Dr. Linghorne had given at the Banting Institute. He reported that he, Dr. Ham and Dr. Williams had met with Dr. Linghorne once during the year to discuss the project with him and to attempt to outline a specific objective for the further prosecution of the research (22nd Mtg., Min. 8). On the basis of the report submitted, however, it appeared that little actual research had been done during the past year. It was felt that Dr. Linghorne has obviously devoted considerable time to the evolution of a concept of bone repair, but that he might have reached an impasse as to the experimental procedures which might best be employed to test his hypothesis.

(ii) DR-23: Dr. C. P. Leblond
Reported by Dr. Copp

"Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements."

A summary and progress report appear in APPENDIX I.

Following presentation of the report, Dr. COPP said that it was his opinion, in which the Committee concurred, that the work being done by Dr. Leblond and his colleagues was of excellent calibre and well deserved the support of the Committee.

(iii) DR-37: Dr. D. H. Jenkins
Reported by Dr. Macdonald

"Continuing studies in electromyographic technique."

A progress report appears in APPENDIX F.

Dr. MACDONALD presented to the Committee the report submitted by Dr. Grainger on behalf of Dr. Jenkins who has now left the University of Toronto. This investigation had not received a grant from the Committee during the year 1956 - 57 but had been carried on by graduate students in partial fulfilment of M.Sc. requirements. It was AGREED that this project might be considered terminated as far as the Committee is concerned, pending receipt of further applications in the field.

(iv) DR-51: Dr. R. L. deC. H. Saunders
Reported by Dr. Lussier

"A study of developing human intradental and peridental vascular patterns by micro-radiographic methods."

A progress report appears in APPENDIX E.

Dr. LUSSIER outlined the work completed during the year and exhibited several excellent X-ray micrographs of the vasculature of the dental pulp. Since Dr. Saunders is on sabbatical leave at the Cavendish Laboratory, no further grant application has been received in connection with this project.

(v) DR-52: Dr. L. F. Belanger
Reported by himself

"The intimate mechanism of mineralization."

A summary and progress report appear in APPENDIX B.

Dr. BELANGER presented, with illustrative slides, a report on the more dental aspects of the extensive studies being carried out in his laboratory on the mechanism of bone and tooth formation. He described the autoradiographic studies he had made, using $S^{35}O_4$, S^{35} methionine and Ca^{45} , and with the aid of slides and drawings delineated the sites and modes of deposition of various minerals in the developing tooth of the rat. He described in detail the "gap" in the enamel organ which, though rarely referred to in the literature, he had found in the molar but not in the incisor. At the site of this gap the ameloblasts change in appearance and there is a massive linear deposition of minerals. He stated that in his opinion this was a natural occurrence, and not an artefact arising from experimental technique.

Following presentation of the report and further discussion with the members of the Committee, the CHAIRMAN thanked Dr. Belanger for his most interesting report.

(vi) DR-57: Dr. G. Nikiforuk
Reported by Dr. Copp

"The organic components of dental tissues."

A summary and progress report appear in APPENDIX J.

Dr. COPP outlined the work done by Dr. Nikiforuk during the past year in determining the organic components of dental tissues by means of chromatography, and commented on its excellence. In the course of the discussion which followed, Dr. BEVERIDGE noted that difficulty had been encountered in removing the ammonia, and suggested that Dr. Nikiforuk might find it worthwhile to try the "dry steam" method which he had found effective.

Since no further application has been received for a grant in support of this project, it is now considered to have terminated. It is understood that Dr. Nikiforuk has made application for support to the National Institutes of Health.

(vii) DR-58: Dr. J. G. Aldous
Reported by himself

"The metabolism of procaine hydrochloride and related compounds."

A summary and progress report appear in APPENDIX C.

Dr. ALDOUS reported that he had not made as much progress as he had hoped during the year because he had been unable to obtain suitable assistance until late in the Fall. He described the experiments which had been carried out with various local anaesthetic preparations and the effect of adding adrenaline and eserine as cholinesterase-inhibitors. It was found that the addition of eserine, a vaso-dilator, increases the absorption of the anaesthetic preparations and does not appreciably affect their potency, but the addition of adrenaline, a vaso-constrictor, limited absorption of the anaesthetic to such an extent that the concentration had to be reduced to one-tenth. Dr. Aldous also pointed out that another variable - the general health of the subject - entered into the determination of the toxicity of the anaesthetic preparations; he had found that liver disease in particular greatly increased the toxic effects of some anaesthetics.

Further experiments are being continued using Primacaine.

(viii) DR-59: Dr. D. H. Copp
Reported by himself

"Effect of severe phosphorus deficiency on bones and teeth."

A summary and progress report appear in APPENDIX N.

Dr. COPP described the experiments he had done to determine the clearance of Ca^{45} and P^{32} from plasma by bone; these involved the withdrawal of blood from the carotid artery of anaesthetized adult rats at a constant rate over a certain clearance period. These kinetic experiments confirmed his earlier findings that the calcium uptake by bones in animals fed on phosphorus-deficient diets was much less than that by teeth, which varied little from the control animals. A method was also developed for calculating bone blood flow.

(ix) DR-60: Dr. F. C. Fraser
Reported by Dr. Barrie

"Studies on the pathogenesis of cleft palate."

A summary and progress report appear in APPENDIX M.

Dr. BARRIE reported on the work done by Dr. Fraser during the year on his study of the occurrence of cleft palate in mice under various prenatal influences. During the discussion which followed, Dr. BEVERIDGE suggested that the foetal abnormalities might not necessarily be induced by dietary deficiencies, as claimed by Dr. Fraser, but might result from the interference with oxidation, such as had been observed in Indians of the Andes.

The CHAIRMAN noted that this had been a terminal grant for this project; Dr. Fraser's work appeared to be more genetic than dental in character, and he had been advised to submit applications for future grants to the Assisted Researches Committee.

(x) DR-61: Dr. A. S. V. Burgen
Reported by himself

"Secretion of protein in saliva."

A progress report appears in APPENDIX A.

With the aid of slides, Dr. BURGEN reviewed the work done on the continuous recording of protein secretion in parotid saliva when the gland was activated by stimulation of the temporal nerve. He commented particularly on the surprisingly high output (amounting to about 720 g/kg/day) of protein following exhaustion of the protein stored in the parotid gland. Radioactive tracers were employed to investigate this phenomenon, and it now appears that this

high output is caused by the duct system of the gland rather than by the acinar cells. Further experiments are planned to test this theory and to determine the nature of this protein.

Following discussion of this interesting development, the CHAIRMAN thanked Dr. BURGEN for his presentation.

(xi) DR-63: Dr. K. J. Paynter (with Dr. A. M. Hunt)
Presented by Dr. Williams

"A histological study of teeth and periodontal tissues of guinea pigs reared on subminimal allowances of ascorbic acid."

A summary and progress report appear in APPENDIX L.

Dr. WILLIAMS described in some detail the work done by Dr. Paynter and Dr. Hunt and stated that by this work the grantees have succeeded in describing for the first time the normal pattern of changes which take place in the growing tooth, e.g. the movement of individual teeth and the enlargement of their shape, etc.

Dr. WILLIAMS also reported that during the course of the work Dr. Hunt, holder of a Graduate Dental Research Fellowship, had shown intelligence and application, and gives evidence of becoming a good research man.

(xii) DR-64: Dr. K. J. Paynter
Reported by Dr. Williams

"Investigations into the nature of cementum."

A summary and progress report appear in APPENDIX K.

The work done on this project was described in detail by Dr. WILLIAMS and it was noted that the grantee hopes to continue his study of cellular and acellular cementum during the coming year.

(xiii) DR-65: Dr. Perreault and Dr. Pelletier
Reported by Dr. Racey

"An evaluation of the disturbances of the dentin formation in the rat incisor following trauma of various origins."

A summary and progress report appear in APPENDIX H.

Following presentation of the report on the work carried out, Dr. RACEY expressed the opinion that the grantees had attempted too broad a programme and might have been better advised to study a part of it in greater detail. It was generally agreed that the research had produced interesting results, but that the grantees had apparently not entirely realized their significance. It was suggested that further investigations might be made to try and determine whether the sensitivity of the teeth to trauma can be attributed to blood flow and circulation in the jaw.

(xiv) DR-66: Dr. K. I. Melville
Reported by Dr. Aldous

"Studies on the mechanisms of gingival changes associated with drug administration."

A progress report appears in APPENDIX D.

Following Dr. ALDOUS' presentation of Dr. Melville's report on the production of experimental gingival hyperplasia, Dr. RACEY said that Dr. Melville's assistant, Dr. Francis, had proved to be a good research worker; it was suggested that Dr. Francis be encouraged to apply for a suitable fellowship which would enable him to work full-time in research and get the necessary experience and training to continue in independent research.

6. Report from Fellowship Holders

(i) Dr. E. M. Madlener - Senior Dental Research Fellow

The CHAIRMAN reported that Dr. Madlener, who had been working under his direction at the University of Toronto, had resigned his Fellowship in December, after six months' tenure. He is now on the staff of the Faculty of Dentistry at the University of Toronto.

Dr. MACDONALD presented Dr. Madlener's report on "Cultural Requirements of Bacteroides nigrescens", which appears in APPENDIX P. The report was well received by the Committee, and it was agreed that, with adequate supervision, Dr. Madlener appears capable of good research. Dr. MACDONALD also reported that, as a teacher, Dr. Madlener had shown considerable improvement during the past year.

(ii) Dr. M. Weiss - Graduate Dental Research Fellow

Following presentation of Dr. Weiss's report on "Proteins in Saliva" (APPENDIX O), work done during tenure of his initial Fellowship in 1955 - 56, his supervisor Dr. BURGEN reported in some detail on the progress made by Dr. Weiss during the past two years. Although he has shown some improvement in his capabilities for research and has technical ability, Dr. BURGEN felt that Dr. Weiss should not be encouraged to make a career of research. His interest in research is intense, however, and Dr. BURGEN felt that he might make a suitable part-time research assistant, particularly if he were concerned with projects more closely related to clinical dentistry rather than to the basic sciences.

7. Action by the Executive Since the Last Meeting

(i) Non-recurring grant to Dr. Saunders

The CHAIRMAN reported that the Executive had been asked by Council for an opinion on the application made by Dr. R. L. deC. H. Saunders for a non-recurring equipment grant of \$5,000 to enable him to purchase and install an X-ray microscope recently developed in England. The Executive had recommended, by letter ballot, that the grant be made if sufficient funds became available; the CHAIRMAN reported that Council had subsequently approved the grant in principle, but it was unlikely that the money could be paid this year since other applications had been given higher priority.

(ii) Travel Grant to Dr. Belanger

The CHAIRMAN reported that the Executive had voted, by letter ballot, to approve Dr. Belanger's application for a travel grant of \$150 to enable him to attend the Gordon Conference in Maryland last summer. Those who attended the Conference reported that Dr. Belanger had contributed greatly to the success of the meeting.

It was noted in passing that Dr. COPP had been elected Chairman of the 1957 Session of the Gordon Research Conference on the Chemistry, Physiology and Structure of Bones and Teeth.

8. Applications for Grants for 1957 - 58

The CHAIRMAN reported that careful consideration had been given to all grant applications at the 15th Meeting of the Executive, held on March 4th, and added that although Dr. Ham had not been able to be present he had forwarded his comments and recommendations. The Executive's recommendations were presented to the meeting for discussion.

(i) Renewals

Grantee	Title and Amount Requested	Action
Aldous, J. G. Dalhousie University	The metabolism of procaine hydrochloride and related compounds. IV & V. DR-58 \$3,100	MOTION Copp-Racey APPROVED \$3,100
Belanger, L. F. University of Ottawa	The intimate mechanism of mineralization. DR-52 \$3,500	MOTION Copp-Beveridge APPROVED \$3,500
Burgen, A. S. V. McGill University	Synthesis and secretion of protein in saliva. DR-61 \$5,930	MOTION Beveridge-Racey APPROVED \$5,930
Copp, D. H. University of British Columbia	Phosphorus deficiency - effects on bones and teeth. DR-59 \$6,284	MOTION Aldous-Racey APPROVED \$6,284
Leblond, C. P. McGill University	Entry of carbohydrates into teeth as shown with the help of radioactive elements. DR-23 \$3,500	MOTION Racey-Copp APPROVED \$3,500

Grantee	Title and Amount Requested	Action
Linghorne, W. J. University of Toronto	Investigation of the mechanisms concerned in the maintenance and repair of the supporting structures of the teeth. DR-22 \$3,300	

The Committee was advised that Dr. Linghorne is expected to make application to another body in Toronto for support both of himself and his project. Following considerable discussion as to the probable amount of actual experimentation to be done during the coming year, it was MOVED by Dr. Copp and SECONDED by Dr. Lussier

THAT \$1,500 be encumbered, to be awarded by the Executive when a specific outline - not to exceed 12 lines - of the work to be undertaken next year has been submitted.

CARRIED

Dr. Linghorne is to be advised, in the event that this grant is made, that it is the Committee's understanding that an application for funds is being made to another body and the grant of \$1,500 is made to ease transition.

Melville, K. I. University of McGill	Studies on the mechanism of gingival changes associated with drug administration. DR-66 \$4,300	MOTION Beveridge-Aldous APPROVED \$4,300
Paynter, K. J. University of Toronto	Investigations into the nature of cementum. DR-64 \$3,400	MOTION Williams-Lussier APPROVED \$3,400

Grantee	Title and Amount Requested	Action
Perreault, G. University of Montreal	Effects of acute and chronic trauma on the pulp and growing dentin in the incisors of albino rats. DR-65	MOTION Racey-Copp APPROVED \$2,595

Since Dr. Pelletier is leaving the University of Montreal, he is no longer regarded as co-grantee. The Committee suggested that Dr. Lussier might provide some direction in connection with this project.

(ii) New applications

MacLean, H. R. University of Alberta	The role of epithelium in the genesis of anomalies of tooth development. \$2,252
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Following discussion, it was MOVED by Dr. Aldous and SECONDED by Dr. Lussier.

THAT the amount of \$2,252 be encumbered, to be released by the Executive when additional information has been received from Dr. MacLean as to what he proposes to do. CARRIED

The Committee expressed concern over the low salary proposed for Dr. Mezl, who is apparently a reputable research man of considerable experience.

McMurchy, K. A. University of Alberta	A study of resorption of enamel, dentin and cementum. \$200	MOTION Barrie- Williams APPROVED \$200
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During consideration of the applications for grants, Dr. Barrie noted that tremendous benefit was being derived by basic science departments who undertake research relating to teeth because they can obtain equipment of general usefulness by means of grants from the Dental Committee. He wondered whether the dental faculties of the grantees' universities were able to make use of these tools and the skill in their operation developed by members of the basic science departments. Dr. COPP replied he was always glad to assist members of other departments by lending available equipment; he felt

that, ideally, Physiology Departments should be common to both medical and dental schools since members of these departments have a responsibility to everyone doing research in the Biological Sciences. Dr. BURGEN agreed with this principle and said that, in the new Physiology Laboratory planned for McGill, space was to be set aside for research in dental physiology if the Dental School will help to maintain it. Dr. COPP felt that investigators in fundamental departments should be glad to help their clinical colleagues and reported that an obstetrician and a pediatrician were presently making use of his laboratory facilities. It was generally agreed among the investigators on the Committee that the ideas brought forward by clinicians were often very stimulating, but they concurred in Dr. BEVERIDGE's view that co-operative effort was needed - clinicians should not expect the basic scientists to solve, alone, the clinical problems which arose.

Discussion then turned to the distinction between dental and medical research. It was suggested that dental research could not at present be restricted only to the study of problems which are of immediate interest to practitioners; Dr. BURGEN felt that there has up to now been such a paucity of fundamental work in the field of dental research that any information derived from basic studies of the mouth will eventually be incorporated into dental practice. It was agreed that research which was primarily clinical in nature should be supported by the Dental Committee only if it is directly related to a dental problem. Dr. COPP pointed out that it was certainly the responsibility of the Dental Committee to encourage the development of dental research, but if in doing so it limited its activities only to the more practical type of problem it would not be sponsoring research but merely paying lip service to public opinion. Dr. BARRIE said that all biological investigators should be encouraged to note the effects of their experiments on the teeth, wherever possible, since apparently they are now often overlooked.

Dr. O'NEIL suggested that rather than leaving the nature of the problem investigated up to individuals, the Committee should initiate research which it feels needs to be done. He inquired about the possible usefulness of preparing a list of projects of interest to the Dental Committee. The CHAIRMAN replied that when the Committee was first established, almost no dental research was being done and this approach had been tried in order to stimulate interest in it. It had not been successful, however, and the CHAIRMAN felt that no investigator could

be induced to undertake research in which he was not interested; he therefore expressed the opinion that the Committee could not initiate research, but could only give those who wished to undertake a project the opportunity to do so.

Dr. O'NEIL also felt that there was not sufficient work being done on human subjects. While he acknowledged that many of the projects on experimental animals led to results which were applicable to man, he was of the opinion that correlation and interpretation of the information obtained by pathologists, physiologists and others would be of great assistance to dental practitioners in solving the problems they encountered in their work. It was generally agreed that dental clinicians should be encouraged to take more interest in fundamental research and, indeed, to do work in the field, as medical clinicians had been doing to an increasing extent since the war. The CHAIRMAN said that at the present time dental clinicians were not sufficiently qualified to do fundamental research; they have to be trained, and he felt strongly that the Associate Committee on Dental Research should do everything in its power to see that they are so trained.

(iii) Non-recurring grant for equipment

In view of the fact that Council may not have sufficient funds to make an award to Dr. R. L. deC. H. Saunders to enable him to purchase the X-ray microscope which he has requested, it was MOVED by Dr. Lussier and SECONDED by Dr. Williams,

THAT the amount of \$5,000 be granted to Dr. Saunders from the Committee's 1957 - 58 allotment for this purpose.

CARRIED

9. Application for Fellowship

- (i) Dr. M. Weiss, to work under Dr. A. S. V. Burgen at McGill University (renewal).

Dr. BURGEN said that Dr. Weiss is at present writing his M.Sc. thesis and that his final estimation of Dr. Weiss' ability depends to some extent on the outcome. It was agreed, however, that Dr. Weiss does not appear to be a suitable candidate for an independent research career and he should therefore not be further encouraged in this direction by a renewal of his Fellowship.

Following further discussion, it was MOVED by Dr. Copp and SECONDED by Dr. Beveridge,

THAT the sum of \$3,000 be encumbered, to be added to Dr. Burgen's grant to enable him to employ Dr. Weiss until March 31, 1958 in the event that he wishes to do so following termination of Dr. Weiss' present Fellowship in June.

CARRIED

10. Application for Travel Grants

The CHAIRMAN presented to the meeting for its consideration applications from Dr. K. J. Paynter, Dr. G. Nikiforuk and Dr. A. M. Hunt for travel grants to help defray the cost of their attendance at the meeting of the International Association for Dental Research in Atlantic City, March 20 - 23, 1957 to present papers on work carried out under the aegis of the Committee. It was MOVED by Dr. Copp and SECONDED by Dr. Racey,

THAT travel grants of up to \$150 be made to each of the three applicants for this purpose.

CARRIED

11. Finances

(i) Financial status of the Committee

The CHAIRMAN circulated a statement of the financial status of the Committee as of 28 February, 1957. It was noted that following payment of the expenses of the 23rd Meeting, sundry duplication charges, and the travel grants noted above, the 1956 - 57 allotment of the Committee would likely be entirely exhausted.

(ii) Estimates for 1958 - 59

It was AGREED that the estimates for 1958 - 59 be left to the discretion of the Executive for consideration in the Fall when the financial picture had perhaps been clarified.

12. Membership of the Committee

The SECRETARY reported that Dr. D. L. Thomson, Vice-Principal and Dean of Graduate Studies at McGill had been chosen by Council as its representative on the Associate

Committee on Dental Research. The Committee felt itself fortunate in this choice and regretted that due to other commitments Dr. Thomson had been unable to attend the meeting.

The CHAIRMAN presented to the meeting a letter from Dr. J. S. Bagnall who expressed his desire to retire from the Committee in view of the fact that his health prevented him from attending its meetings. Following discussion, it was MOVED by Dr. Aldous and SECONDED by Dr. Copp,

THAT the Committee accept, with regret, the resignation of Dr. Bagnall.

CARRIED

Consideration was then given to the replacement of retiring members of the Committee. The CHAIRMAN reported that the terms of office of Dr. Lussier, Dr. Barrie, Dr. Williams, Dr. Ham, Dr. Burton and Dr. Macdonald expired in 1957. He pointed out that in accordance with Council policy, the terms of members who had served only one three-year term could be renewed, on the recommendation of the Committee, for a further period of three years. It was MOVED by Dr. Copp and SECONDED by Dr. Beveridge,

THAT a recommendation be forwarded to Council that the terms of Dr. Lussier, Dr. Barrie, Dr. Williams and Dr. Macdonald be renewed for a three-year period to expire March 31, 1960.

CARRIED

Nominations were then requested for replacements for Dr. Ham and Dr. Burton, who had already served for a second term, and for Dr. Bagnall. The CHAIRMAN drew attention to the fact that consideration should be given to a fair geographic representation and also to the equitable division of the membership between dentistry and the basic sciences. Following the close of nominations and on vote, it was AGREED

THAT a recommendation be forwarded to Council that Dr. K. J. Paynter, Dr. David Scott and Dr. C. Castaldi be appointed to the Associate Committee on Dental Research for a three-year term to expire March 31, 1960.

It was AGREED that letters be written to Dr. Ham, Dr. Burton and Dr. Bagnall, acknowledging the Committee's indebtedness for the valuable service they had rendered during their terms of office.

The CHAIRMAN also reported that Dr. J. B. Marshall wished to resign as active Secretary of the Committee and it was to be recommended to Council that Miss D. J. Wright of his office be named Secretary in his place. Dr. MARSHALL assured the Committee that he would still take an active interest in its activities and would look forward to attending its meetings in an ex officio capacity. The Committee expressed its appreciation of the assistance Dr. Marshall had given during his term of office, both as Secretary of the Committee and in an advisory capacity.

(ii) Membership of the Executive

The CHAIRMAN reported the proposal of the present Executive that its membership be increased by one. This was agreeable to the Committee and it was MOVED by Dr. Copp and SECONDED by Dr. Racey,

THAT Dr. Lussier and Dr. Beveridge be named to the Executive for 1957-58.

CARRIED

13. Publications of Grantees

The CHAIRMAN reported that reprints of the following papers had been received by the Secretary during the past year from grantees of the Committee:

RAE, J. J., Shemilt, H. M. and Clegg, C. T. Ammonia production in saliva. I. J. Dent. Res. 34: 886-888, 1955.

CRAVEN, A. H. Growth in width of the head of the Macaca rhesus monkey as revealed by vital staining. Am. J. Orthodontics 42: 341-362, 1956.

KROPP, B. N. Preparing single or serial sections of calcified bones and teeth. Stain Technol. 31: 253-261, 1956.

Hunter, H. A. and MACDONALD, J. B. The effects of single injections of soluble components of "fusospirochetal" materials in experimental animals. J. Dent. Res. 35: 4-8. 1956.

MACDONALD, J. B. Mixed infections of the oral cavity. J. Norwegian Dental Assoc. April 1956.

MACDONALD, J. B., Sutton, R. M., Knoll, M. L., Madlener, E. M. and Grainger, R. M. The pathogenic components of an experimental fusospirochetal infection. J. Infectious Diseases 98: 15-20, 1956.

MACDONALD, J. B., Sutton, R. M. and Knoll, M. L. The production of fusospirochetal infections in guinea pigs with recombined pure cultures. J. Infectious Diseases 95: 275-284, 1954.

It was noted that only five reprints of each paper reporting work done under grants from the Committee need be submitted to the Secretary instead of the 25 previously required.

14. Summer Employment for Dental Students

Dr. LUSSIER asked the Committee to consider the feasibility of offering summer scholarships to undergraduate dental students, with a view to arousing their interest in research. He felt that although support is being given at the graduate level in the form of grants and fellowships, this had little effect on the stimulation of interest in research in undergraduates. Dr. LUSSIER suggested that summer research studentships valued at \$600 - \$800 be awarded to one student from each of the five dental schools, to enable them to work in research laboratories between academic sessions. He further suggested that at the end of the summer, the students be asked to submit a brief report on their work and on the department in which they had worked, and a similar report be obtained from the departments concerned on the students. Dr. LUSSIER felt that in this way, undergraduate dental students might be made aware of the opportunities offered in research, opportunities which they can rarely see under the present circumstances of relative inactivity in research evident in most dental schools.

Considerable discussion ensued. Dr. BEVERIDGE felt that the proposed stipends were not realistic, since they would not offer sufficient attraction to offset the advantages of other employment possibilities open to the students. Dr. COPP felt that the idea was very sound and represented one of the best ways of stimulating research in the field of dentistry. He was of the opinion that the best students in the class should be encouraged to participate in such a scheme, and that this would be possible if the remuneration were sufficiently attractive. Dr. WILLIAMS suggested that rather than confining the plan to one student from each dental school, a total of five students might be given awards, regardless of their schools.

Dr. MARSHALL said that such studentships would be outside the scope of the National Research Council at the

present time, since the Council makes awards only at the post-graduate level. He suggested, however, that the plan might be operated as an employment opportunity, on a basis similar to that now in operation at the Council in order to recruit people for its own laboratories, and with comparable rates of pay, e.g. \$265. per month, initially, with nominal increases allowed for experience.

It was the opinion of the Committee that such employment might be offered to dental undergraduates regardless of the amount of academic training they had received, because the earlier one can interest a student in research the greater the likelihood of his continuing interest in it. It was suggested that from the administrative standpoint such students might, for the time being, most feasibly be employed in the laboratories of the Committee's grantees, who could if necessary be given supplementary grants to cover the necessary expenditure involved.

It was MOVED by Dr. Lussier and SECONDED by Dr. Beveridge,

THAT the Executive be directed to explore the possibilities of employing undergraduate dental students in the laboratories of dental grantees during the summer months, and be empowered to act thereon if possible.

CARRIED

15. Dental Research Associateships

The CHAIRMAN reported briefly to the Committee on the Medical Research Associateships offered by the Council and suggested that the establishment of similar Associateships in the dental field would give great encouragement to dental graduates interested in research. He felt that at present dental graduates could see little opportunity for a rewarding career in research and turned instead to the more lucrative career of private practice. The Committee agreed that while there was no likely candidate for such an Associateship at the present time, the availability of such positions would undoubtedly arouse considerable interest on the part of dental graduates in the advantages of further research training. It was MOVED by Dr. Copp and SECONDED by Dr. Beveridge,

THAT a recommendation be forwarded to Council that approval be given, in principle, to the awarding of Dental Research Associateships on terms analogous to those governing Medical Research Fellowships.

CARRIED

16. Date of Next Meeting

It was AGREED that the date of the next meeting be set by the Chairman and the Secretary, when the dates of other Council Committee meetings have been arranged.

The meeting adjourned at 1:10 p.m., March 5, 1957.

Project No.: D.R. 61

Amount of Grant: \$3,400.00

Since the report of last year, very considerable progress has been made in the analysis of protein synthesis and secretion in salivary glands. It will be recalled that the continuous record of protein concentration in parotid saliva when this gland was suddenly activated from rest by stimulation of the temporal nerve showed two peaks of protein concentration. The first peak appeared within a few seconds of starting stimulation (up to 3% protein) and was labelled P₁. This first outburst of protein was followed by a minimum and then a second peak which appeared between 45 seconds and 2 minutes after the onset of stimulation. This second peak was labelled P₂. Analysis of the relation of P₁ and P₂ to the frequency of stimulation and the interval between periods of stimulation of the gland showed that these two peaks were of different origin and were controlled by different parameters. The amount of protein in P₁ was entirely independent of the rate of stimulation and depended only on the rest interval since the last period of stimulation. Thus, if the interval between periods of stimulation was less than 1 minute, hardly any P₁ appeared. At intervals longer than 1 minute P₁ increased proportionately, at least up to periods of an hour and a half. There was never any sign that P₁ could be exhausted, that is when successive periods of stimulation up to 40 or 50 in number had been applied to the gland over a period of 4, 6 or even 7 hours, P₁ still appeared and was not diminished in size. P₁ accumulated at the rate of about .7 mg. protein per minute. The properties of P₂ were quite different. In the first place the peak concentration attained in P₂ was dependent on the frequency of stimulation. Thus, as the frequency was increased the concentration of protein increased more or less linearly. This means that in this range the output of protein from the gland in P₂ goes up very steeply with the frequency of stimulation. In most glands the rate of saliva secretion reached a maximum when the frequency of nerve stimulation reached 15 to 20 per second.

APPENDIX A

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: Secretion of protein in saliva

Grantee: Dr. A.S.V. Burgen,
Department of Physiology, McGill University

Project No.: D.R. 61

Amount of Grant: \$3,400.00

Since the report of last year, very considerable progress has been made in the analysis of protein synthesis and secretion in salivary glands. It will be recalled that the continuous record of protein concentration in parotid saliva when this gland was suddenly activated from rest by stimulation of the temporal nerve showed two peaks of protein concentration. The first peak appeared within a few seconds of starting stimulation (up to 3% protein) and was labelled P_1 . This first outburst of protein was followed by a minimum and then a second peak which appeared between 45 seconds and 2 minutes after the onset of stimulation. This second peak was labelled P_2 . Analysis of the relation of P_1 and P_2 to the frequency of stimulation and the interval between periods of stimulation of the gland showed that these two peaks were of different origin and were controlled by different parameters. The amount of protein in P_1 was entirely independent of the rate of stimulation and depended only on the rest interval since the last period of stimulation. Thus, if the interval between periods of stimulation was less than 1 minute, hardly any P_1 appeared. At intervals longer than 1 minute P_1 increased proportionately, at least up to periods of an hour and a half. There was never any sign that P_1 could be exhausted, that is when successive periods of stimulation up to 40 or 50 in number had been applied to the gland over a period of 4, 5 or even 7 hours, P_1 still appeared and was not diminished in size. P_1 accumulated at the rate of about .7 mg. protein per minute. The properties of P_2 were quite different. In the first place the peak concentration attained in P_2 was dependent on the frequency of stimulation. Thus, as the frequency was increased the concentration of protein increased more or less linearly. This means that in this range the output of protein from the gland in P_2 goes up very steeply with the frequency of stimulation. In most glands the rate of saliva secretion reached a maximum when the frequency of nerve stimulation reached 15 to 20 per second.

Almost invariably, however, the concentration of protein went on rising as nerve stimulation frequency was increased at least up to 30 - 40 per second. Secondly, P₂ was subject to exhaustion, that is successive periods of stimulation at the same frequency yielded lower and lower peak values until P₂ could be almost completely exhausted and in this case the protein pattern after commencement of stimulation would show a first peak of P₁ followed by a low plateau of a level of .6 to 1.5 g/L protein. If sufficient rest interval were allowed before the next period of stimulation, P₂ would to some extent recover but the degree of recovery was always relatively small although it was proportionate to time. Our preliminary interpretation of this data suggested that P₁ was derived from cells in which there was normally only a very small or negligible pool of protein, but that continuous synthesis of protein went on in these cells and that during a rest period sufficient protein accumulated to produce the P₁ proportionate to this interval. P₂, on the other hand, was primarily derived from protein stores in the gland which could be readily exhausted and, in fact, integration of the amount of protein that could be removed up to the time at which P₂ had disappeared gave values of the order of 40 or 50 g. of protein per kilogram of parotid gland. This would amount to about one-half of the total protein in the gland. The recovery of P₂ with longer rest intervals represented a resynthesis of part of the store of protein present in the resting gland. The rate of reappearance of protein in P₂ after a rest agreed well with the values obtained for protein synthesis in the gland by Anrep by whole gland analysis, and by others on the tissue slice work. What could not be accounted for was the relatively high rate of protein output that occurred in the steady state left after P₂ had been exhausted. This represented a protein secretion of about 0.5 g. of protein per kilogram of gland per minute or about 30 g/kg./hr. or 720 g/kg./day. This rate of excretion is so high and so out of proportion to the known rate of acquisition of storage protein by the gland that we considered that this probably had a different origin that might have something in common with P₁. Our first idea was that perhaps this did not represent protein synthesized by the gland at all, but merely protein transmitted through the gland from the plasma directly into the saliva. In order to test this hypothesis we prepared plasma albumin labelled with Iodine 131 on the tyrosine group. This was labelled protein prepared only with minimum amounts of iodine averaging 1 atom of iodine per mol of protein. After intravenous injection of this labelled albumin, labelled protein appeared in the saliva within 20 seconds and reached a steady concentration almost immediately. In the phase where P₂ is exhausted the specific activity, that is the amount of radioactivity per gram of protein in the salivary protein was almost as high as that

in the plasma protein showing that almost all the saliva protein was either plasma protein or was almost completely derived from it. In order to investigate this problem in more detail methods were devised for studying the radioactivity in single drops of saliva secreted by the gland. The drops of saliva were collected on a moving strip of filter paper and fixed in place by heat coagulation and washed to remove any non-protein iodine. The spots were then punched out with a metal punch and counted individually in an end-window counter. These experiments showed that the output of radioactive protein from the gland gave a high peak corresponding exactly in time course with P_1 followed by a low plateau level corresponding with the plateau protein concentration found after P_2 was exhausted. Further, if the radioactive protein was injected into the central end of the lingual artery so that it reached the parotid gland in high concentration in a transient form, a similar transient appeared in the saliva radioactivity. This transient rose extremely steeply so that the time from the first appearance of radioactive protein until the maximum concentration was reached was of the order of only 20 - 30 seconds. This suggests that there can only be a very small reservoir volume of labelled protein within the gland and virtually excludes the passage of the radioactive protein through the main acinar cells. On other grounds, mainly the comparison of time course with the secretion of radioactive inorganic iodine and potassium strongly suggested to us that the P_1 was a function of the duct system of the gland and not of the main gland system. We think it probable that almost all the radioactive protein secretion is a function of the high columnar duct system of the gland and not of the acinar cells, although some later evidence suggested that a small component possibly different in nature and corresponding in time to P_2 may be a product of the acinar cells. This remains to be investigated in detail.

We were next concerned with the nature of the protein secreted in the saliva containing radioactivity. Was it simply plasma albumin or had it been converted in this very short period into a specific salivary protein? To study this we have developed methods of separating the proteins on a column following the general method described by Porath and also standard paper electrophoresis as described for gastric proteins by Jerzy Glass. First we showed that the injected radioactive albumin behaved exactly like normal albumin. No change had occurred in the protein as a result of the iodination process and the iodine was firmly bound in a chemical bond. Secondly, electrophoresis of parotid saliva has showed as a rule three protein components. These all are slower moving than the albumin and when albumin is

added to lyophilised saliva proteins it can be clearly distinguished by its mobility. Our present results with column, paper and Tiselius electrophoresis have demonstrated that there is little or no protein with the same properties as albumin in parotid saliva. The upper limit we can set at the present time is about 5% of the total protein and this is quite insufficient to account for the amount of radioactive protein that appears in saliva. At the present time then we believe that the radioactive protein is converted into salivary protein at an extremely rapid rate by the duct cells. This conversion still remains to be proved and a great many further experiments are needed before we will be satisfied about it. A further experiment along these lines has been done by the injection of a specific normal plasma constituent, namely lysozyme, to see whether this had any pronounced effect on the saliva lysozyme concentration. The object of this experiment was to see whether a specific protein could penetrate the salivary gland intact and appear in the saliva. So far these experiments have not shown that the saliva lysozyme concentration follows in any way the plasma concentration. This result confirms in a quantitative way some qualitative experiments performed by Florey some years ago.

To summarize, we are beginning to reveal some of the details of protein secretion by salivary gland and we have demonstrated an entirely unsuspected route of protein formation in the salivary duct system which, under many circumstances, can be more important than that of the protein produced in the acinar cells. We do not know at the present time what are the relative compositions, that is in the purely chemical sense, or functions of the different kinds of proteins secreted by the two systems. We have developed methods for studying the secretion and synthesis of these protein groups independently and we are in the process of developing methods for isolating the fractions and characterizing them.

APPENDIX B

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: The intimate mechanism of mineralization
Grantee: Dr. L.F. Belanger
Project No.: D.R. 52 Amount of Grant: \$2,465.00

SUMMARY OF WORK

Part VII: The mineralization of enamel appears different in the incisors and molars of young rats when viewed in the light of Ca45 autoradiography and also by micro-incineration.

In the incisor, the process is regular and progressive.

In the molar, the initial slow process is followed by massive mineralization of young enamel when the organic matrix stops being secreted. This is followed by progressive general mineralization of the completed organic structure.

The various phases in the life of the enamel organ are often morphologically indicated by the presence of successive semi-circular gaps.

Before eruption, the enamel receives its minerals via the enamel organ.

Part VIII: The intake of radiocalcium by specific regions of the skin, eye, cartilage and mucous glands has been demonstrated with the help of integrated autoradiographs.

Most of the calcium that enters the hair follicle is found in the keratogenous zone. In the skin and eye, it appears that Ca45 has entered by bio-synthesis while in the cartilage and mucous glands, the intake of labelled Ca might represent cation adsorption by the sulfopolysaccharides.

The autoradiographic record of Ca45 follows the distribution of the blue/white dust in the spodograms of skin, cartilage and mucous glands.

Part IX: Magnesium deficiency produces defects in the long bones of rats which are more apparent at 14 days than at 28 days of deficiency duration. Abnormal bone growth and temporary atrophy of the epiphyseal plate have been described. A new histophotometer has permitted comparative studies of autoradiographs of S35 methionine labelled epiphyseal plates. These have revealed interesting topographical data in the normal animals and a decreased proportional uptake of radio-methionine by the epiphyseal cartilage of the deficient animals.

PROGRESS REPORT

Experimental Procedure and Results

Part VII: Ref. attached copy of manuscript submitted for publication in the Journal of Dental Research.

Part VIII: Ref. attached copy of manuscript entitled: "The entry of Ca^{45} into the skin and other soft tissues of the rat", in print with the Journal of Histochemistry and Cytochemistry.

Part IX: The effect of magnesium deficiency on the growth and composition of mineralized tissues of the rat.

PURPOSE

With the help of specific staining and autoradiography of S^{35} distribution in the tissues, to study (1) the synthesis of proteins in various tissues (2) blood formation and destruction.

EXPERIMENTAL PROCEDURE

Three groups of rats (5 animals in each group, averaging 40-50 gms) were placed on a casein diet containing less than 1 mg. of Magnesium per 100 gms of diet (Sup. No.1). An equal number of comparable controls were fed the same diet to which had been added 0.6 gms $\text{Mg SO}_4 \cdot 7 \text{H}_2\text{O}$ per 100 gms of diet mechanically blended in for 2 hours.

The mixture moistened with distilled water was offered ad libitum. The amount of diet consumed was recorded daily. Body weight was recorded twice per week. A sample of blood was obtained from each animal by cutting the tail twice per week in order to determine total red cell count, total white cell count, differential and haemoglobin. The animals of the third group (28 days on diet) were injected with a tracer dose of methionine S^{35} 4 hrs. before sacrifice. These procedures were carried out by Mrs. Anita Jakerow during the 4 months of her employment under the above-mentioned grant. All tissues were fixed in either neutralized alcohol-formaldehyde or Bouin's picroformol, for histological, histochemical and autoradiographic studies.

A fourth group of 2 animals were fed the deficient diet for 14 days and were then fed the control diet for 14 days in order to observe potential recovery from the deficiency symptoms. These animals were studied as above.

RESULTS

(1) Growth and Feeding Characteristics: A study of the growth chart (Sup. No.2) shows for the Mg. deficient animals, a retardation of growth beginning at day 7 and maintaining itself throughout the experiment. The recovery group has gained weight almost immediately and has almost reached up to the controls at 28 days.

(2) Daily food intake: The food intake chart (Sup. No.3) has shown a concurrent decrease in the deficient group. The two animals which were fed the control diet after 14 days of deficiency, have shown a considerably increase of food consumption almost immediately after the return to the normal diet.

(3) Gross symptoms: Apart from growth and feeding differences, the Mg. deficient animals have shown the following symptomatology: At 4 days, they exhibited hyperactivity and nervousness. The skin, particularly in the region of the nose, vibrissae, eyes and ears as well as over the feet became intensely red. At 10 days, the deficient animals were highly excitable, frightened by noise. At day 12 some developed convulsive seizures. One developed diarrhea and was sacrificed immediately. At day 13, one animal died after a convulsive seizure. The fur of all deficient animals was ruffled at that stage. Subcutaneous hemorrhages particularly around the nose and eyes appeared. At day 14, patchiae appeared along the tail. The fur was lost in patches. Another animal was lost in convulsions on day 15. All animals showed bloody secretions at nasal and buccal orifices. After that period, the vascular and nervous symptoms seemed to decrease towards the 28 day period.

The two animals placed on control diet on day 15 were apparently the most affected. They showed marked general improvement almost immediately. After 2 days, the testis were in the scrotal position, the fur looked better, the skin lesions were healing. At 28 days, these recovery animals looked like the controls.

(4) Histology: Schrader and al ('37) state that in view of the dramatic physiological manifestations of Mg deficiency, there is "surprisingly little histopathology". Changes have been reported in the skin (Schrader and al, '37; Barren, '48; Watchorn and McCance, '37) in the kidney (Watchorn and McCance, '37); Lowenhaupt and al '50; Dick and Prior, '51), in the central nervous system (Bird, '49; Van Reen and Pearson, '53), in the liver (Schrader and al, '37); in the connective tissue (Duckworth and al, '41; Dick and Prior, '51) and principally in the

cartilage, bones and teeth (McCollum and Orent, '31); Becks and Furuta, '41; Klein, Orent and McCollum, '35; Watchorn and McCance, '37, '42; Duckworth and al, '41; Dick and Prior '51).

Metabolic changes in relation to protein synthesis (Menaker and Kleiner, '52), tissue respiration (Westphall, '51), alkaline phosphatase, diphosphopyridine nucleotidase (Van Reen and Pearson, '53), ATP ase (Cantarow and Schepartz, '54) activities as well as in the synthesis of the thyroid hormone (Honorato and Moya, '46) have been reported.

Findings related to haematology, histopathology and autoradiography of soft tissues have been reported to the Medical Division (MP. 174).

HARD TISSUES

Teeth: Changes in the newly formed enamel and dentine as well as in the pulp chamber of molars and incisors have been observed. A detailed study of these tissues is being carried out presently.

Cartilage: Atrophy of the epiphyseal plate of long bones has been previously reported (Dick and Prior '51). In the present material, this atrophy was remarkable at 14 days after the onset of deficient diet (Supplement 4, figs. 1, 2). At 28 days however, the thickness of the plate has become comparable to that of controls (Supplement No. 4, figs. 3, 4 and Suppl. No. 5). However, the underlying mineralized cartilage and newly formed spicules appears crowded together (Supp. No. 4, figs. 3 and 4), possibly due to a decrease in the width of the bone at that level.

Bone: In one animal of the 14 day stage, the epiphyseal plate of the lower extremity of the tibia (Supp. 6, fig. 7) appeared disrupted and poorly functional. A large cellular mass consisting of cells that looked like fibroblasts and osteoblasts was seen in place of the normally growing spicules.

Three animals (out of 5) exhibited at this 14 day stage, abnormal development of poorly mineralized bone in the middle of the diaphysis (Supp. 6, fig. 8).

At 28 days, none of the abnormal growth were observed inside the bone shaft but rapidly growing newly formed bone appeared in the periosteal surface of the mid diaphysis (Supp. 6, figs. 5, 6). In one instance, the bone was accompanied by several small islands of cartilage (fig. 6) never present in such a location of course, under normal conditions.

AUTORADIOGRAPHY

Comparative autoradiographs of the epiphyseal plate were studied with the help of an excellent histophotometer (Supp. 7 figs. 9, 10) also developed in the course of this year. A portion of the epiphyseal plate autoradiograph projected onto a screen at 160 diameters has revealed 4 zones with graded densities. When these zones were brought in turn to the small opening of the photo cell (fig. 10), the actual optical density of the area could be recorded. Comparisons indicate that the zone of young cartilage is more intense than the zone of hypertrophic cartilage which in turn is more intense than the underlying calcification zone (Supp. No. 9) in normal animals. By comparison also, the underlying area of bone spicule formation is midway in optical density between zone A and B in the controls.

Autoradiographs of sections of deficient tibiae (figs. 11, 12) have shown a lower optical density for all zones of the cartilage (Supp. No.9), pointing to a lower uptake of methionine. The area of the spicules however was darker, possibly on account of the apparently larger number of spicules per area (figs. 3,4).

Regional histophotometry has revealed that the region of the plate which is midway between the peripheral ring of cartilage replacement (notch of Ranvier) shows the most intense rate of methionine incorporation possibly related to the maturation of the cells (Supp. No.9). A peak in the same position has been found at the level of bone formation, possibly related to a larger turnover of cartilage in that central area (Supp. No.9).

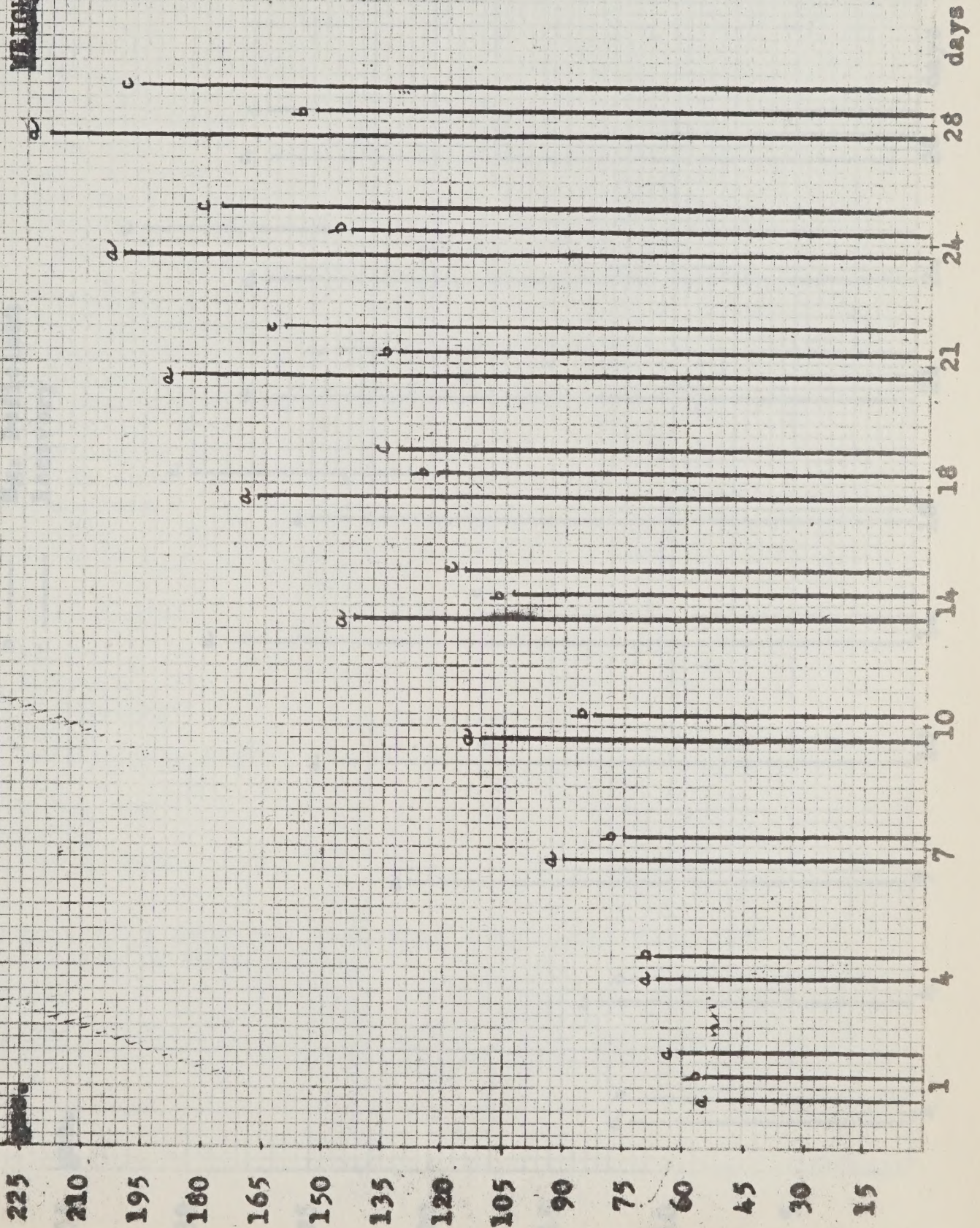
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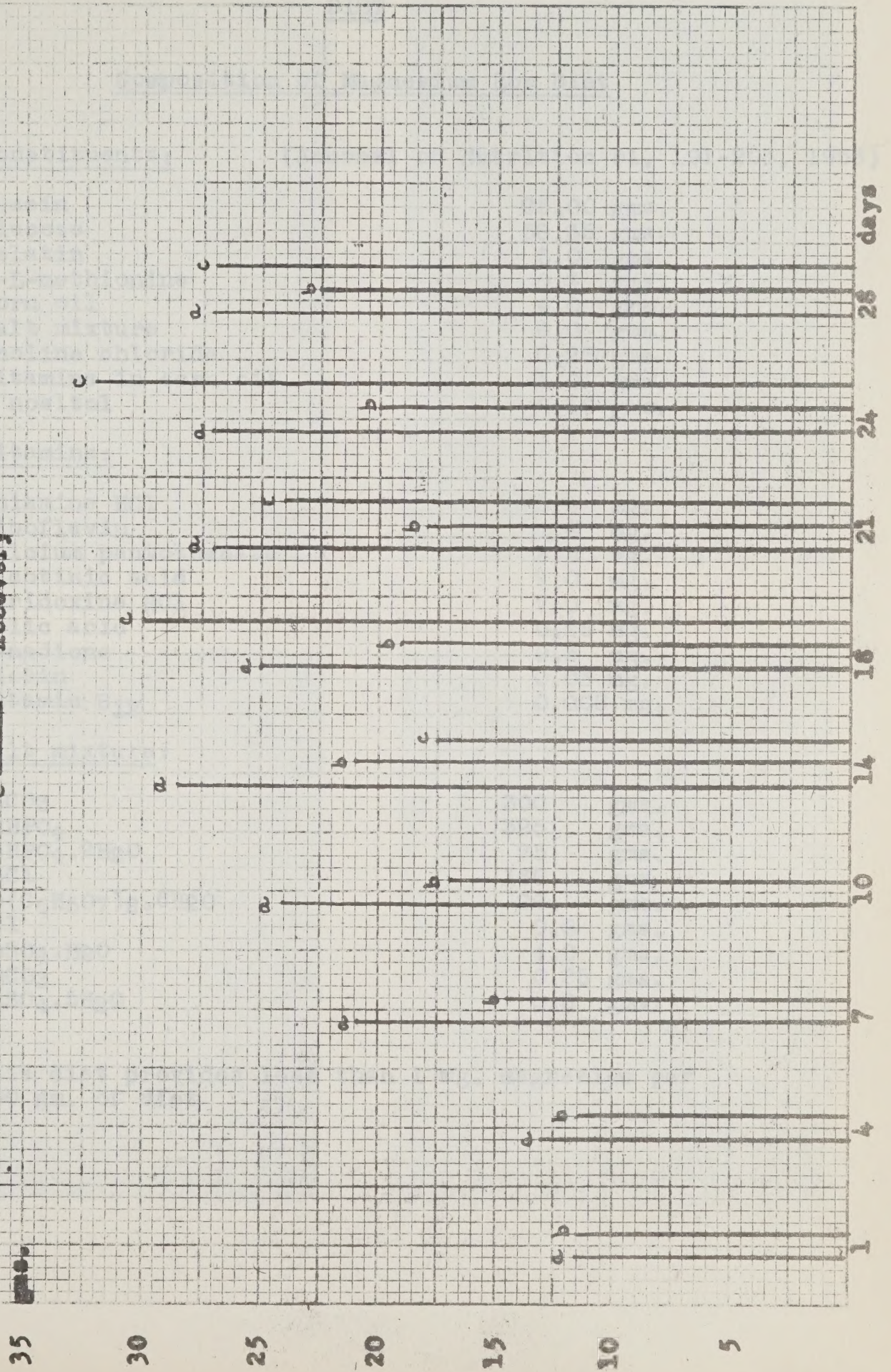
Supplement No. 1 (DR. 5.2)

WEIGHT



FOOD INTAKE (WET DIET)

a — Control
b — Mg. Deficient
c — Recovery



DIETComposition of Magnesium Low Diet

Constituents: (Journal of Nutrition 51, 191-203, 1953)

Casein	25.00 gms.
Glucose	58.35 gms.
Gelatin	5.0 gms.
D-L-methionine	0.3 gms.
Corn oil	4.0 gms.
Salt mixture	6.0 gms.
Choline chloride	0.25 gms.
Vitamins in corn oil	1.0 gms.
I nositol	0.10 gms.

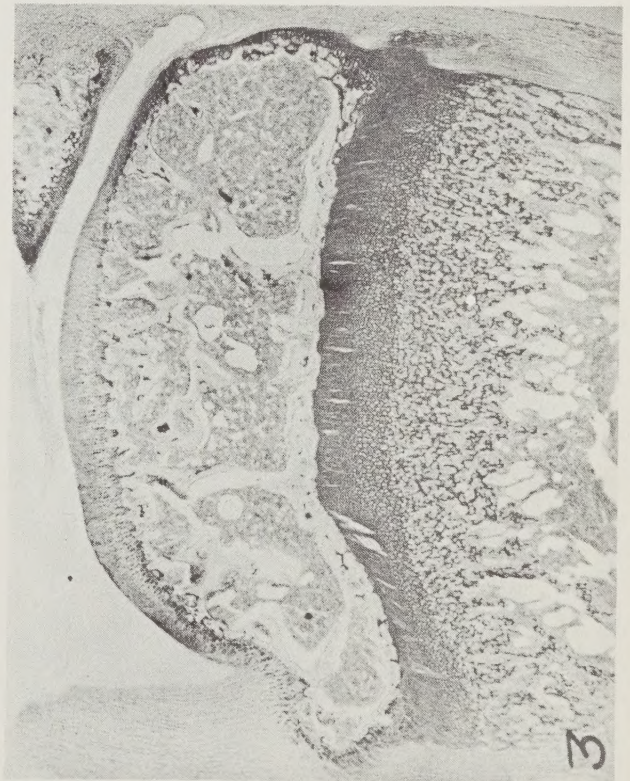
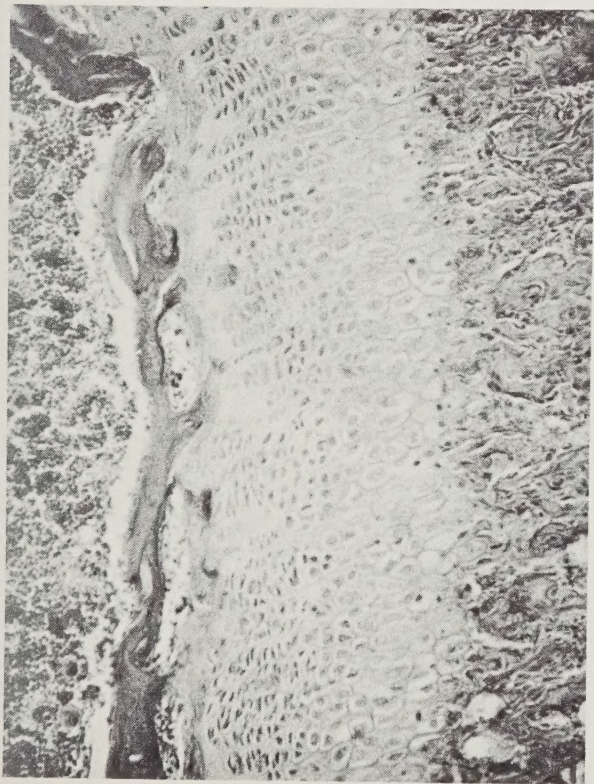
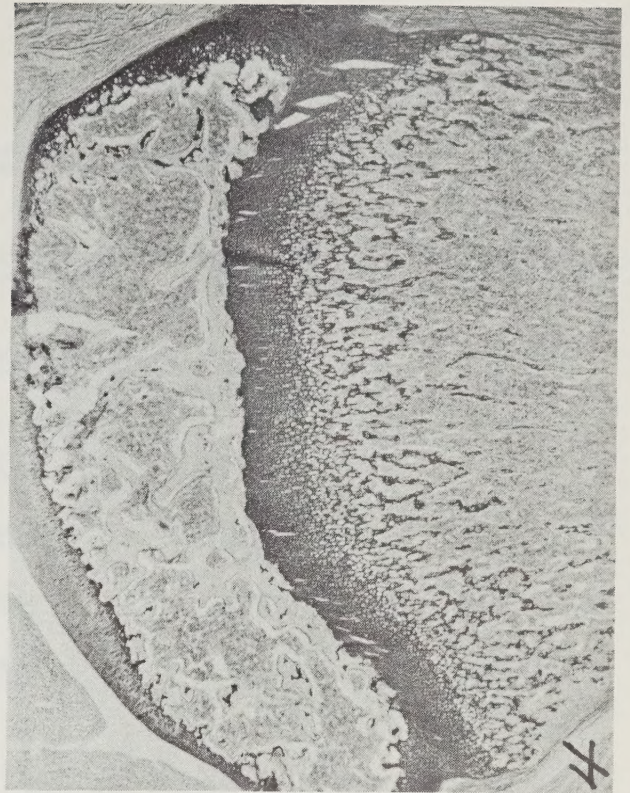
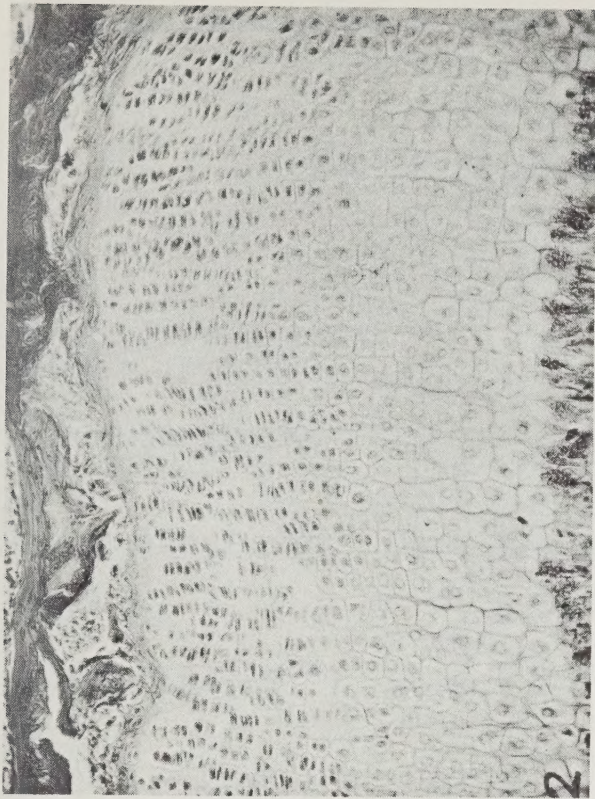
Vitamins:

Thiamine HCl	0.6 mg.
Riboflavin	0.6 mg.
Calcium pantothenate	2.5 mg.
Nicotinic acid	7.0 mg.
Pyridoxine HCl	7.0 mg.
Folic acid	0.25 mg.
Menadione	0.5 mg.
Biotin	0.03 mg.
Vitamin B ₁₂	0.005 mg.

Salt mixture:

CaCO ₃	300 gms.
K ₂ HPO ₄	325 gms.
CaHPO ₄ ·2H ₂ O	75 gms.
NaCl	168 gms.
Fe(C ₆ H ₅ O ₇) ₂ ·6H ₂ O	28 gms.
KCl	0.8 gms.
MnSO ₄ ·H ₂ O	4.0 gms.
ZnCO ₃	0.25 gms.
CuSO ₄ ·5H ₂ O	0.3 gms.

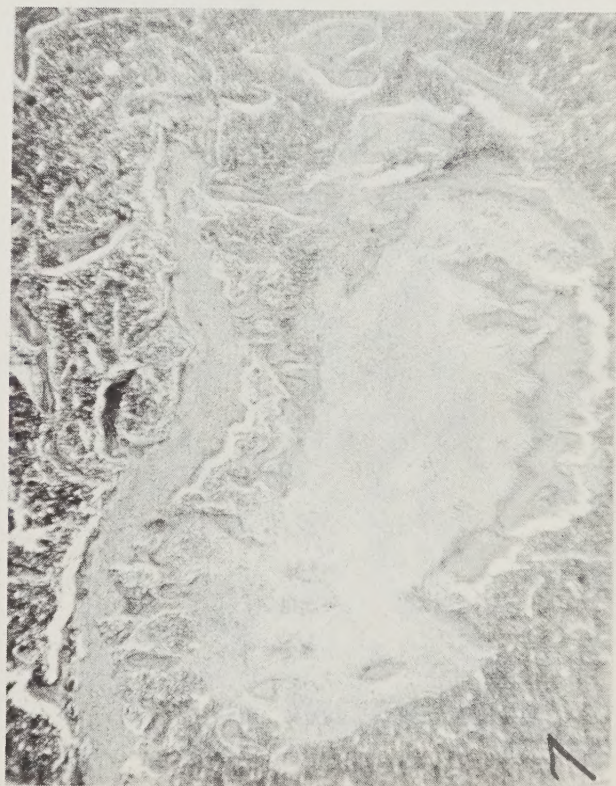
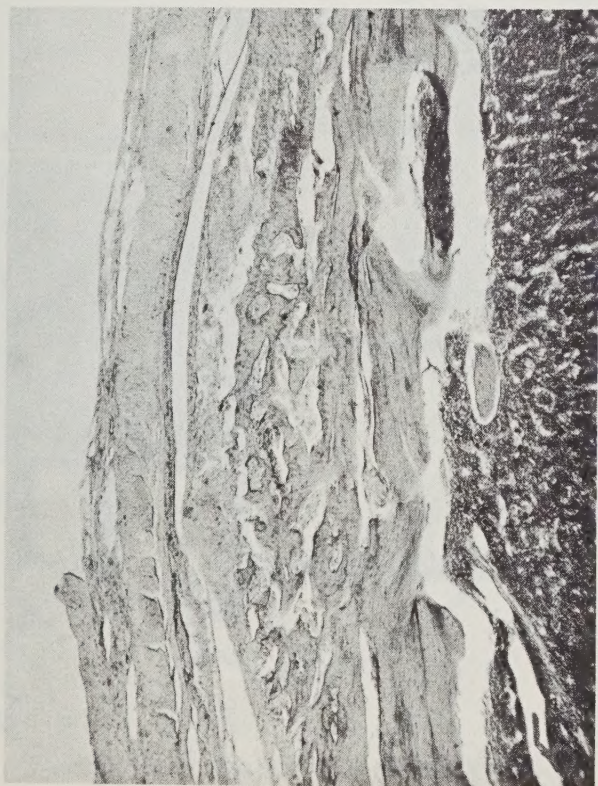
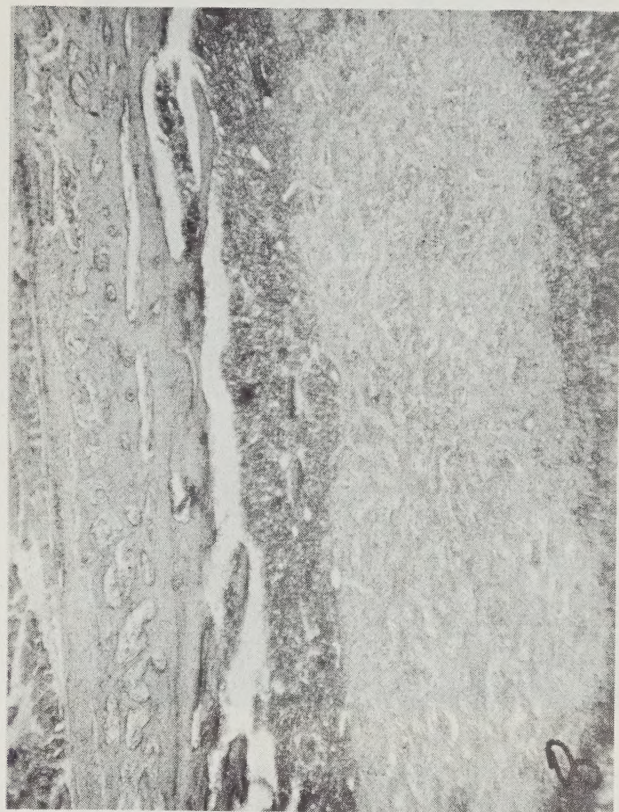
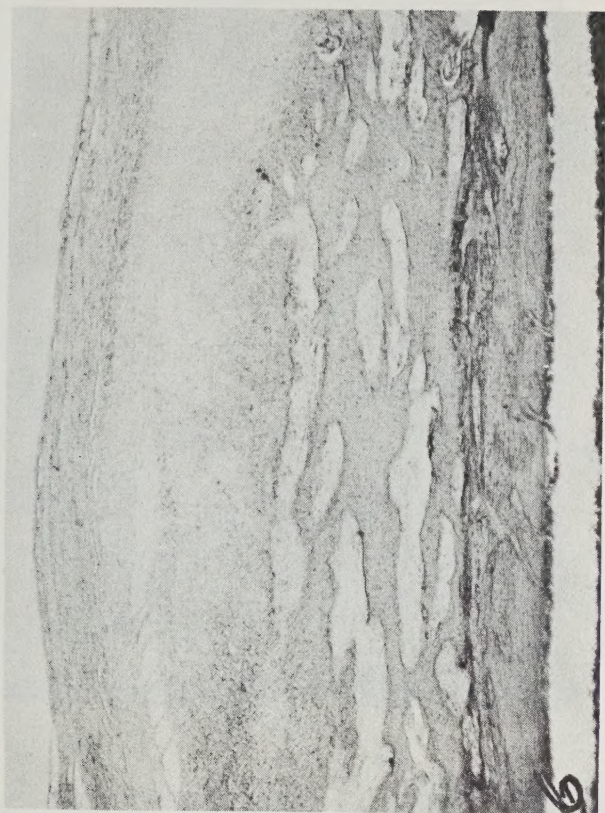
This diet provides less than 1 mg. magnesium per 100 gm. of diet



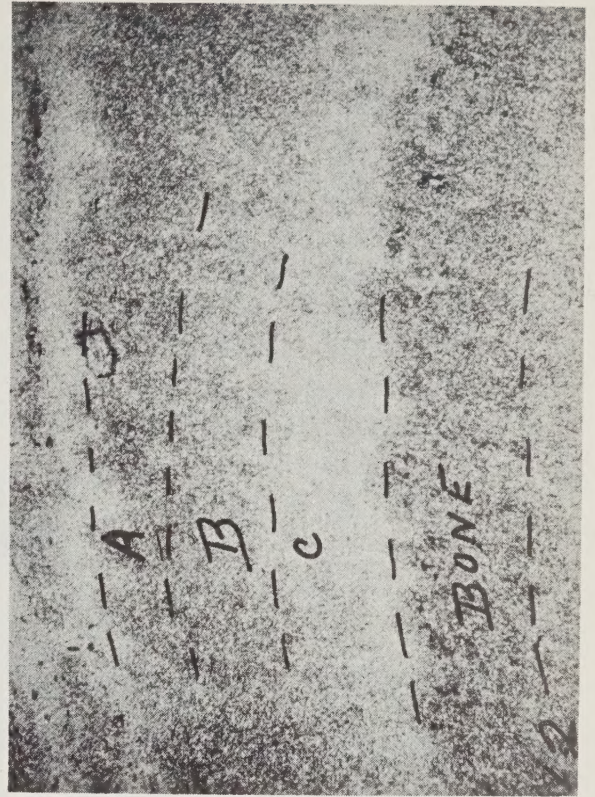
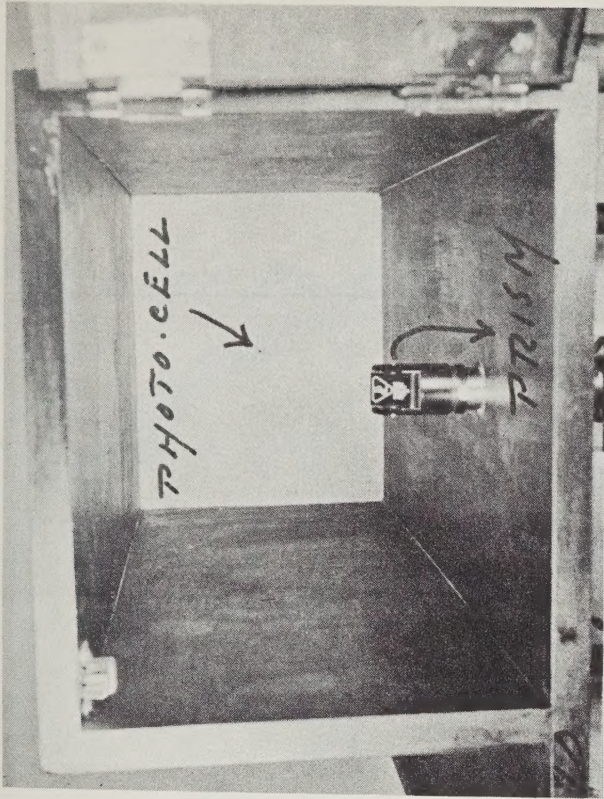
Supplementary No. 4 (DR-52)

Supp. No. 5Thickness of Epiphyseal Plate

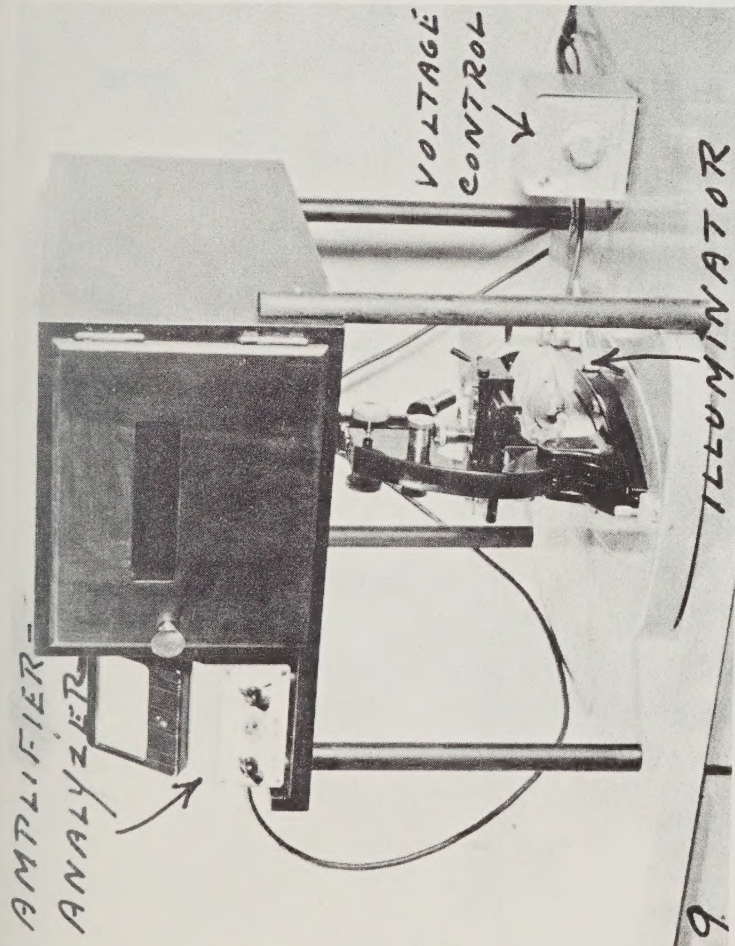
		<u>Control</u>	<u>Mg. Def.</u>	<u>Difference</u>
Stained epiphyseal plate (Wright)		450 micra	434 micra	
Autoradiographs of $S^{35}O_4$		300	334	
Autoradiographs	Cart A & B	233	220	
of	" C	58	63	
Methionine (S^{35})	Bone	266	143	54%



Supplement No. 6 (DR. 52)



Wasp. No. 1 (1-195-7-12) DK-52



Supp. No. 8LIGHT ABSORPTION

		<u>Control</u>	<u>Mg. Def.</u>	<u>Difference</u>
Cartilage	A	35 (✓ - 0.6)	26 (✓ - 3.3)	-26%
	B	25 (✓ - 1.2)	21 (✓ - 2.7)	-16%
	C	17 (✓ - 1.0)	13 (✓ - 1.0)	-18%
Bone		29 (✓ - 1.5)	33 (✓ - 1.0)	✓14%

Tibia, Rats of 50 days
(Diet 28d); Methionine S³⁵

(4 hrs. s.c.)

carti lage {	A--	34 (19)	32 (22)	43 (32)	39 (28)	29 (27)
	B--	24 (18)	26 (20)	28 (20)	25 (19)	21 (24)
	C--	20 (12)	17 (12)	20 (14)	14 (10)	14 (13)
Bone--		27 (30)	28 (32)	31 (40)	25 (36)	

{ A=Young cartil.
Cartilage { B=Hypertrophic
 { C=Calcified

THE MINERALIZATION OF RAT ENAMEL IN THE LIGHT OF CA⁴⁵
AUTORADIOGRAPHY AND MICRO-INCINERATION

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INTRODUCTION

The mechanism of mineralization of enamel is still mysterious. It is generally agreed that it takes place over a prolonged period and the term maturation is utilized to describe the changes which occur after the secretion of the primary substance. According to Leicester ('49) who summarizes the opinions of the majority of authors, once mineralization of the matrix begins, it continues as a steady, gradual process. There is, however, a group of investigators (Mellenby '29, Diamond and Weinmann '40) who describe a "secondary calcification" as a separate event taking place in the portion of enamel which has achieved full width. The present series of observations, combined with previously reported data (Bélanger and Leblond '50, Bélanger '52, '53, '54, '55, '56) cast some light on this aspect of the problem as well as on that of the matrix-minerals relationship and on the behavior of the enamel organ.

MATERIALS AND TECHNIQUES

Three hamsters (*Cricetus auratus*) of 4 days of age (av. weight 4 gms.), 5 white rats (Sprague-Dawley) of 6 days (av. w. 16 gms.) and 2 rats of 10 days (Av. w. 27 gms.) were injected subcutaneously with a single dose of $3 \mu\text{c/gm Ca}^{45}$ as calcium chloride in HCl with a high specific activity. These suckling animals were returned to their mothers and sacrificed at intervals of 2 hrs. to 6 days afterwards. The dissected tissues were fixed in formaldehyde-ethanol for 48 hrs. (Bélanger '50), dehydrated in graded ethanol and embedded in low viscosity nitrocellulose for sectioning. Integrated autoradiographs of 12μ sections were obtained by the coating-inversion technique (Bélanger-Leblond '46, Bélanger '50, '52). Other sections were incinerated by the method of Policard-Scott (McClung '50) and examined by dark field microscopy.

OBSERVATIONS

The autoradiographic record of entry and transit of radiocalcium in dentine was essentially similar to that previously reported in incisors of normal pigs (Bélanger, Lotz, Visek and Comar '54) and also comparable to the observations on young rats, recently published by Kumamoto and Leblond ('56).

The entry of Ca^{45} into the enamel of the 4 days old hamsters and 6 days old rats was generally in form of a diffuse band of lower intensity than that of dentine, extending more or less along the whole length of the substance and located at some distance from the surface. This wide band showed also a gradient of intensity towards the dentino-enamel junction.

In the two posterior cusps of the first molar in the rat of 6 days, there was a more intense and wider reaction at the apex, facing the small ameloblasts. This zone was also recognized in animals sacrificed 2, 4 and 6 days after the introduction of tracer.

With time (up to 6 days), the localization of the radioelement seemed to change very little, except that the new increment of enamel had made the layer thicker, giving the impression that the labelled area was more deeply located.

The observations of early entry (2 hrs) in the molars of the rat of 10 days were more varied. At that age, there are pronounced degrees of "maturation" between the first and the second molar, even between the cusps of the same tooth. In the young tissue of the second molar (fig. 1, M2a), the picture previously described could be recognized in the new distal portion of the tissue.

Looking towards the apex, we see the autoradiographic record decrease and eventually disappear to reappear at the very top, much stronger. In the first cusp of the first molar (fig. 1, M1a), these two events can also be recognized. Here though, the apical zone is wider than in M2a. In the second and third cusps (fig. 1, M1b and c), the apical localization is the only one visible. There is a gradient in this secondary image from a to b to c: the autoradiographic record is progressively more intense and covers a wider area of the enamel. In M1b and c (fig. 1) it extends distally in form of a thin band located at a regular distance from the surface. This extreme distal extension of the secondary image corresponds to the presence of a bridge of short ameloblasts (fig. 1, arrows) raised in a hemi-circle above the surface of the enamel and previously described as "gap" (Bélanger '56). Apart from these areas of greater intensity, the enamel has shown a general low intensity record. Dentine and bone have also produced a general low intensity record except at the accretion surfaces where the image is considerably darker (fig. 1).

When the sections were pre-incubated for 3 hrs. in a formic-citrate bath a pH 4.9, counts with the Geiger-Mueller apparatus have been reduced to background; also none of the afore mentioned autoradiographic localizations have been obtained.

Microincineration:- The ash picture (spodogram) revealed practically no minerals in the odontoblasts. Their lateral limits however were well marked by parallel bands of white ash apparently related to the intercellular cement and previously observed in the teeth of pigs (Bélanger, Lotz, Visek and Comar, unpublished). By opposition, the ameloblasts exhibited a cellular content of fine ash, apparently more abundant in the large cells distal to the gap. In these, the ash seemed evenly distributed, while in the smaller ameloblasts, above the gap, the ash appeared only in the basal portion of the cells.

In the young animals, the superficial portion of the enamel of the molar teeth (probably pre-enamel, Chase '35, Leblond, Bélanger and Greulich '56), appeared devoid of minerals while the inner portion showed a progressive concentration towards the dentino-enamel junction.

In the rats of 10 days, a similar pattern was observed in the youngest distal portion of the tissue (figs. 2 and 3). At the level of the gap, a high concentration of minerals appeared in form of a band occupying presumably the zone of young enamel and appearing interposed between two parallel bands of lower mineral concentration (figs. 2 and 3). The initial distal band of minerals enlarged progressively and eventually joined with this secondary deposit. Past the area of the gap towards the apex, the

mineral deposit occupied progressively the whole surface of enamel and appeared to be increasing in concentration (fig. 3) to eventually match in density, the uniformly highly mineralized dentine.

The spodogram of the incisor tooth of the upper jaw appeared different. The ash deposit, originally a thin line at the dentino-enamel junction, increased steadily in thickness and in concentration until it occupied the whole width of the enamel.

DISCUSSION

The interpretation of autoradiographs is subject to recognition of atomic interexchange and actual accretion by bio-synthesis. Evidence has been obtained previously (Bélanger '53) to the effect that when mineralized sections of bones and teeth are placed in a solution of $\text{Ca}^{45}\text{Cl}_2$, radioactive intake is greater in areas of lesser mineralization. In the present material, the radioactive component is entirely labile in weak acid and most likely mineral in greatest part. The autoradiographic pattern obtained from the teeth of young animals, indicate a possible correlation between total mineralization and intensity of the autoradiograph in the enamel but not in the dentine. In that tissue as well as in bone, numerous reports (Leblond, Wilkinson, Bélanger and Robichon '50; Comar, Lotz and Boyd, '52; Bélanger, Lotz, Visek and Comar '54; Kumamoto and

Leblond '56) have shown the accretion surfaces as the most active sites, indicating that most of the radioactive substance present is being bio-synthetized locally. This view has been confirmed by experiments in which the deposition of organic large molecules actually required a complex biochemical mechanism (Bélanger '54, '55, '56). Consequently, it may be assumed that the enamel picture represents largely accretion.

In the early stage of enamel formation, there appears to be mineral deposition taking place sometime after the formation of organic matrix. On the other hand, in the molars of the 10 days old rat, particularly in the first molar, it seems that a secondary and more intense process of mineralization begins later at the level of young enamel. This in turn is followed by deposition of minerals throughout the whole tissue.

The spodogram of different parts of the molar tooth enhances the autoradiographic demonstration of a triphasic operation.

At the dentino-enamel junction of the youngest, most distal portion of the tissue, a thin band of ash is visible. This band increases slowly in width and density. At a distance from the original formative site, the enamel reveals the presence of another deposit of minerals, linear in appearance (figs. 2 and 3) and located between two zones of lower mineral content. Further up towards the apex of the cusp, this band is joined by increasing minerals from

underneath and disappears as a visible entity as the mineral concentration increases progressively towards the apex and towards the surface of the tooth.

In previous work, evidence of this secondary phase of mineralization has been obtained without being fully recognized. In a paper dealing with autoradiographic mapping of P^{32} intake by the teeth (Bélanger '52), we had observed in the enamel of the first molar of a 6 days old rat, "an area of higher intensity at the junction of approximately the outer one third with the inner two thirds", persisting two days after the introduction of tracer. In the same paper, a silver nitrate reaction on the first molar of a 4 days hamster produced "two distinct zones, one at the dentino-enamel junction, the other one near the surface of the enamel". Furthermore, the experiments with Ca^{45} in vitro intake have demonstrated inside the enamel of human molars, zones in which the mineralization was apparently lesser than that of the surface (Bélanger '53).

All these observations on the molar teeth are in favour of the opinion expressed by Mellenby ('29) and by Diamond and Weinmann ('40) to the effect that mineralization of enamel is the result of a double operation. The fact that the second phase of mineralization begins opposite the semi-circular gap, (figs. 1, 2 and 3) appears significant. This feature of the enamel organ has been originally described by Marsland ('51) as marking the end of matrix formation and the beginning of calcification. Such areas

of raised ameloblasts have been observed by us in different portions of the enamel organ: (1) immediately before the beginning of organic secretion (Bélanger '55). (2) At the end of secretion of the organic substance labelled with $S^{35}O_4$ (sulfomucoprotein); this site corresponds also to the cessation of incorporation of S^{35} methionine (Bélanger '56, enamel protein). This area corresponds also to the cessation of production of alkaline phosphatase by the enamel organ (Bélanger '55). Finally, this second and most frequently observed location corresponds to the onset of the presently described secondary phase of mineralization (figs. 1, 2 and 3). (3) A gap has also been seen occasionally at the end of the secondary phase of mineralization, at a point where the whole substance of enamel appears to be recipient to mineral deposition. (4) Finally, more or less large areas of detached epithelium may appear anywhere along the enamel organ as the result of histological technique. These last mentioned artefacts can be distinguished from the functional sites by the appearance of the ameloblasts (Bélanger '55).

The existence of these sites where the ameloblasts change in appearance, where certain functions begin or cease, indicate clearly that the enamel organ is a multiphasic structure which proceeds by steps towards the formation of enamel. In the molars of 10 days old rats, three episodes

In the incisor, the process is regular and progressive.

In the molar, the initial slow process is followed by massive mineralization of young enamel when the organic matrix stops being secreted. This is followed by progressive general mineralization of the completed organic structure.

The various phases in the life of the enamel organ are often morphologically indicated by the presence of successive semi-circular gaps.

Before eruption, the enamel receives its minerals via the enamel organ.

ACKNOWLEDGMENTS

The author is indebted to Mrs. Cécile Bélanger for skilled technique and to the Associate Committee for Dental Research of the National Research Council of Canada for financial assistance. The radiocalcium was purchased from Atomic Energy of Canada Limited.

DESCRIPTION OF FIGURES

Fig. 1: - Integrated autoradiograph (unstained)

of the 1st and 2nd molar, upper jaw, from
a 10 days old, sacrificed 2 hrs. after an
injection of $\text{Ca}^{45}\text{Cl}_2$. x32

Fig. 2: - Spodogram of part of a section of cusp Mlb
(fig. 1). The limits of the gap and the
dentino-enamel junction have been outlined.
x100

Fig. 3: - Spodogram of part of a section of cusp Mlc,
treated as above. x100

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THE ENTRY OF CA⁴⁵ INTO THE SKIN AND OTHER SOFT TISSUES
OF THE RAT: AN AUTORADIOGRAPHIC AND SPODOGRAPHIC STUDY

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INTRODUCTION

The presence of a variety of minerals (K, Na, Mg, Ca, Fe, Cu, Zn) in the epidermis has been demonstrated by chemical methods (Carruthers and Suntzeff '45) and by micro-incineration (Kooyman '35, McCardle, Engman and Engman '43). The techniques of chemistry have the advantages of qualification and quantitation of the elements. Micro-incineration reveals the intimal distribution, unfortunately without specificity (Bourne '51, Lison '53, Montagna '56) except maybe in its recent utilization along with electron mobilization (Scott and Packer '39). Difficulties in appreciating specifically the optical picture (spodogram) has led to publications of contradictory reports (Smith and Parkhurst '49; Lansing and Opdyke '50). Intriguing total ash variations in relation to age, disease (McCardle, Engman and Engman '43; Cowdry, Carruthers and Suntzeff '48) and species (Carruthers and Suntzeff '53) have been described. Differences in "exchange behavior" of the epidermal calcium of normal and tumoral tissue

of the mouse (Lansing, Rosenthal and Kamen '48) have also been reported.

In our previous Ca^{45} experiments dealing with mineralization of bones and teeth (Bélanger, Lotz, Visek and Comar '54; Bélanger, in press), no record of soft tissue intake was observed on account of the small doses of tracer and the short exposures. However, when demineralized sections containing arteries (Bélanger, Burke, Tremblay and Bélanger '54) cartilage and mucous glands, (Bélanger '55) were soaked in a highly active solution of $\text{Ca}^{45}\text{Cl}_2$, more or less intense autoradiographic records of radiocalcium intake were obtained. Variations from the normal pattern were observed in ageing arteries and cartilage (Bélanger, Burke, Tremblay and Bélanger '54) in rickets (Bélanger '55) and in fluorine intoxication (Bélanger, Lotz, Visek and Comar, unpublished). Recently, Johnson (Comar '55) reported a constant "concentration of grains over intercellular matrix" of cartilage.

Such results led to the present attempt to obtain autoradiographs of Ca^{45} from the skin and other soft tissues of animals injected with Ca^{45} . A large dose of radioactive material had to be given and the animals were not expected to survive more than a few hours.

MATERIALS AND TECHNIQUES

Two rats of 10 days of age (av. weight 27 gms) were injected under the dorsal skin with an equal single

dose of 0.2 ml. of a solution of CaCl_2 in HCl , containing 0.758 mc/ml Ca^{45} . They were both sacrificed 2 hours after introduction of the tracer. At that time, both animals had an area of discoloration at the site of injection; one exhibited cyanosis of the extremities, tremor and breathing difficulties. The dissected tissues (skin of the face, eye, soft tissues of the neck, knee joint) were fixed in formaldehyde ethanol at pH 7.8 for 48 hours, (Bélanger '50), dehydrated in graded ethanol and embedded in low viscosity nitrocellulose or paraffin for sectioning. Integrated autoradiographs of 7 to 10 μ sections were obtained by the coating-inversion technique (Bélanger-Leblond '46; Bélanger '50, '52). Other sections were incinerated by the method of Policard-Scott (McClung '50) and examined by dark field microscopy.

OBSERVATIONS

Unfortunately, a systematic survey of all tissues and organs was not undertaken and the present report will be concerned only with the structures which had previously demonstrated a relatively high in vitro intake (Bélanger '55).

After exposures of 1 to 3 months, autoradiographs of the skin (fig. 1 EP) and also those showing the wet, keratinized mucosa of the mouth and the oesophagus, have revealed a weak, uniform picture corresponding to the innermost portion of the tissue. At the level of the follicles

of small hair (fig. 1 B, K) and vibrissae, there was a gradient of intensity; the region of the hair bulb showed a reaction comparable to that of the surface. Higher up, the keratogenous zone (Giroud and Leblond '51) appeared as most active. High power observations of cross sections of the large tactile hairs have revealed that the radioelement is definitely concentrated in the hair and the immediately adjacent inner root sheath. Above the keratogenous zone, the hair revealed practically no radioactivity.

The eye showed considerable variations in the autoradiographic map of Ca^{45} : the outer portion of the sensory retina was most intense (fig. 5 R); the reaction seemed to decrease progressively towards the inner media. The ciliary body (fig. 5 CB), the cornea and the surface of the lens (fig. 5 L; capsule ?) produced remarkable records as compared with the lens itself, the outer coats of the eye and the optic nerve which revealed very minor blackening of the emulsion.

The cartilage of the trachea (fig. 4) as well as that of the head of long bones has revealed a relatively high level of activity as compared to other soft tissues.

In the trachea (fig. 4) the radioelement has appeared as uniformly distributed throughout. In the cartilaginous extremity of the long bones (distal extremity of the femur and proximal extremity of the tibia), regional differences of intensity were observed: at the articular surfaces, the reaction was minimal; then it seemed to increase to a

maximum at the level of the hypertrophic cells. Mineralized cartilage was of course most intense.

The epithelial lining of the nasal cavities and that of the trachea (fig. 4) produced a record of moderate intensity; also the glands containing mucous.

Other tissues such as the thyroid gland (fig. 4), skeletal muscle, could be recognized as slightly above autoradiographic background.

The micro-incinerated preparations of skin revealed a double layer of ash localized at the inner and outer limits of the epidermis and separated by an empty space. The mucosal linings of the mouth and the oesophagus were comparable to the epidermis except that the outer layer of ash appeared less dense.

The snodogram of the hair follicle, particularly the region of the bulb observed in dark field, was a thing of beauty which unfortunately a black and white photograph (fig. 2) could not justly represent. Against the dark background, the ashes of that area appeared as rounded formations varying in size and colour; the majority of the larger spheres were a brilliant yellowish-white. Some were orange-red; small particles dispersed throughout the larger ones formed a blue, dusty background. Higher up, the larger white-yellow and orange elements decreased in number while the blue dust became more prominent (fig. 1). In the keratogenous zone, only the blue dust was seen. It definitely reproduced the shape of the hair and its

immediate neighbour, the inner root sheath (fig. 1). At the periphery of the hair shaft, there appeared to be a greater concentration of the salt, producing a brilliant white border. A concentration of brilliant white ash was present in place of the hair, above the keratogenous zone.

Sections of this material have been soaked in a formic-citrate bath at pH 4.9 for 1 hour at room temperature before being submitted to micro-incineration. The spodogram of such sections (fig. 3) revealed that the minerals had disappeared in greatest part. The innermost region of the skin (stratum germinativum and stratum spinosum) and the hair bulb (fig. 3) now appeared a dull brownish-red, while the stratum corneum and the keratogenous zone of the hair follicle (fig. 3) shined in a bluish-white light; the individual particles in these areas did not seem to have changed either in size or concentration.

In the spodogram of the eye, a heavy layer of ash (fig. 6) seemed to represent the region of the rods and cones and that of the bipolar elements. Inwards, the ganglionic zone showed isolated masses of ash corresponding to individual cells. The external retina and the outer coats appeared practically ashless. The spodogram was not modified by the acid bath.

The mucous glands produced an interesting ash pattern; there was a brilliant yellow-white border at the periphery

of the acini and ducts (basement membrane ?); the distal cytoplasmic portion of the cells contained some bluish-white dust while the apical secretory portion was apparently ashless.

Spodograms of the cartilage of the head of the long bones have already been observed (Bélanger, Lotz, Visek and Comar, unpublished) under conditions of health and disease. In the normally growing rat, pig and human, blue/white ash represents the matrix. The relative amount of ash is lowest at the articular surface; it increases progressively towards the plate to reach a maximum at the level of the hypertrophic cells. In demineralized sections, it decreases rapidly in the subjacent area of the empty lacunae. In the trachea of young rats, the ash is also blue-white and increases slightly in concentration from the periphery towards the center of the cartilage piece.

DISCUSSION

It appears from the observations reported above, that it is possible to trace Ca^{45} into soft tissues. It may be argued that the conditions of dosage utilized in the present experiments are not physiological and that they may have produced radiation damage. On the other hand, it is evident that for an experiment of such short duration, the radiation effects have not prevented the entry of the

radioelement into the various tissues.

In the interpretation of autoradiographs of isotopic intake, another question must be raised; that of atomic inter-exchange and actual accretion or bio-synthesis. Previous work dealing with Ca^{45} intake by sections of tissue in vitro (Bélanger '53), casts some light on that problem. While cartilage, mucous glands and the wall of arteries have produced clear pictures of intake, the epidermis, hair and the retina have shown much less activity after similar treatment.

It is thus possible to ascribe to a biological operation, some of the records obtained in the present series (figs. 1, 4 and 5). The presence of Ca^{45} in the epidermis and hair and also that recorded in the mucosal membranes of the mouth and oesophagus seems to be related to the formation of keratin. The most intense record of radioactive calcium is in specific growth areas (fig. 1) and not necessarily in the zones of largest mineral concentration demonstrated by spodography (figs. 2 and 3). The previously reported calcium intake by the epidermis of normal and carcinogenic mice (Lansing, Rosenthal and Kamen '48) might be reinterpreted in the light of autoradiography. The fact that Ca^{45} enters into the same area of the hair follicle (keratogenous zone) where methionine and cystine become incorporated (Bern, Harkness and Blair '55; Bélanger '56) might indicate a relationship between

the sulfur-containing proteins and the calcium of the skin.

The calcium of mucous glands and that of cartilage appears to be related to the sulfomucopolysaccharides (mucoitin sulfate, chondroitin sulfate) contained in these tissues (Bélanger '55). Since it has been demonstrated that in cartilage, the sulfated polysaccharide is synthesized by the cell and then secreted into the matrix (Bélanger '54, Amprino '55), it is possible to think along with Newman, Boyd and Feldman ('52) that such substances act as cation binders and may retain calcium as it is made available through the tissue fluids.

Finally, the distribution of salt in the epidermis and hair before and after a bath in weak acid (figs. 2 and 3) indicate clearly that a bright white ash should not be hastily interpreted as representing calcium or magnesium. When spodograms and autoradiographs were compared, it seemed that the distribution of the radioactive calcium followed the pattern of the acid resistant blue/white dust (figs. 1 and 3) exclusively.

On the other hand, reports in the literature which ascribe blue ash to sodium and potassium (Scott '33, Lansing and Scott '42) should be received with caution.

It appears from the present study that the blue/white appearance might well represent only different concentrations of the same substance, as postulated by Mason ('31).

In the cartilage and mucous glands as well as in the skin, the acid resistant blue/white dust (small particles) seems to represent calcium. In the retina, the yellowish-white appearance of the ash seems to indicate a complex mixture. High concentrations of minerals have already been reported in neurones of the central nervous system (Ca, Fe, Bourne '51) particularly before differentiation has been completed.

SUMMARY

The intake of radiocalcium by specific regions of the skin, eye, cartilage and mucous glands has been demonstrated with the help of integrated autoradiographs.

Most of the calcium that enters the hair follicle is found in the keratogenous zone. In the skin and eye, it appears that Ca^{45} has entered by bio-synthesis while in the cartilage and mucous glands, the intake of labelled Ca might represent cation adsorption by the sulfopolysaccharides.

The autoradiographic record of Ca^{45} follows the distribution of the blue/white dust in the spodograms of skin, cartilage and mucous glands.

ACKNOWLEDGMENTS

The author is indebted to Mrs. Cécile Bélanger for skilled technique and to the Medical Division of the National Research Council of Canada for financial aid. The radioactive calcium was purchased from Atomic Energy of Canada Limited.

DESCRIPTION OF FIGURES

- Fig. 1: Unstained integrated autoradiograph of a cross section of hairy skin of a 10 days old rat, sacrificed 2 hrs. after an injection of radiocalcium: EP, epidermis; K, keratogenous zone; B, hair bulb. x 57.
- Fig. 2: Spodogram of a celloidin section adjacent to that in fig. 1 x 100.
- Fig. 3: Spodogram of a section as in fig. 2, soaked for 1 hour in formic-citrate. x 100.
- Fig. 4: Unstained integrated autoradiograph of a cross section of the trachea and thyroid from a 10 days old rat sacrificed 2 hrs. after an injection of radiocalcium. x 34.
- Fig. 5: Unstained integrated autoradiograph of a section of the anterior portion of the eye from the animal described in fig. 4: R, retina; CB, ciliary body; L, lens. x 34.
- Fig. 6: Spodogram of a celloidin section of the eye adjacent to that in fig. 5. The heavy layer of ash represents the rods and cones and the bipolar layer. The ganglionic zone underneath, shows isolated clumps of ash representing the ganglionic elements. The external retina and outer coats are ashless. x 100.

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APPENDIX C

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: The metabolism of procaine hydrochloride
and related compounds

Grantee: Dr. J.G. Aldous,
Dept. of Pharmacology, Dalhousie University.

Project No.: D.R. 58 Amount of Grant: \$3,000.00

SUMMARY OF WORK

The results of intradermal wheal tests in guinea pigs show that when absorption is limited by the addition of a vasoconstrictor the potency of certain local anesthetics may be increased by a cholinesterase inhibitor. Augmentation of potency is only possible when the local anesthetic is one which is readily hydrolyzed by cholinesterase. Whereas both diffusion (absorption) and metabolism (hydrolysis) are factors in determining the potency of anesthesia, the latter is probably more important when the question of systemic toxicity arises.

Oxycaine^R hydrochloride was added to the list of local anesthetics whose metabolic characteristics have been investigated. The toxicity of this drug, determined as the maximum rate of metabolism in the unanesthetized rabbit, is slightly higher than that of procaine. It is not readily hydrolyzed by pseudo-cholinesterase and appears to diffuse from blood to brain with greater ease than does procaine.

duced considerably the potency of anesthesia. The account for these observations: (a) eserine is a cholinesterase and hastens absorption from the site of anesthesia and (b) Procaine is not rapidly hydrolyzed by pseudo-cholinesterase and therefore the presence of eserine is expected to influence anesthesia to any great extent. The latter fact is demonstrated clearly in section 4 and Table 1, where, in solutions containing adrenaline or ephedrine, absorption, eserine does not change the potency of anesthesia.

A preliminary report of this work was presented at the Maritime Dental Association Convention, Halifax, 1956.

PROGRESS REPORTSection A - Local Anesthesia Tests^{*}

Studies previously reported to the Committee have indicated that Procaine and Unacaine, although characterized by almost identical i.v. toxicities, have greatly difference s.c. toxicities. This difference appears to be due to the more rapid hydrolysis of Unacaine by pseudocholinesterase. The rates of diffusion from blood to brain are very similar. Ravocaine resembles Procaine in the rate of its enzymatic hydrolysis, but unlike the other two drugs, it appears to diffuse into the brain fairly readily.

Since enzymatic hydrolysis greatly reduces the anesthetic action of Unacaine, but not of Procaine and Ravocaine, it seemed worthwhile testing the influence of a cholinesterase inhibitor on the potency of anesthesia of these three drugs.

Solutions of the three local anesthetics containing eserine and/or adrenaline were injected intradermally into guinea pigs and the animals tested for local anesthesia according to the method of Bulring and Wajda (J. Pharm. 85: 81, 1945).

Results

Table 1 records the results using Procaine with various additives. It will be noted in this and subsequent tables that when adrenaline is present in the solution the concentration of local anesthetic must be reduced to one-tenth of that without adrenaline.

The addition of eserine to the solution of Procaine reduced considerably the potency of anesthesia. Two facts account for these observations: (a) eserine is a vasodilator drug and hastens absorption from the site of deposition and (b) Procaine is not rapidly hydrolyzed by pseudocholinesterase and therefore the presence of eserine would not be expected to influence anesthesia to any great extent. The latter fact is demonstrated clearly in sections C and D of Table 1, where, in solutions containing adrenaline to limit absorption, eserine does not change the potency of anesthesia.

^{*} A preliminary report of this work was presented at the Maritime Dental Association Convention, Halifax, June 1956.

Table 1

The anesthetic properties of Procaine and additives.

Values represent anesthetic potency (with standard errors) where 36 equals 100% anesthesia for 30 mins.

Solution A - Procaine
 Solution B - Procaine + eserine (25 µg/ml)
 Solution C - Procaine + adrenaline (20 µg/ml)
 Solution D - Procaine + eserine + adrenaline

Concentration per cent.	Anesthetic potency	
	A	B
2.0	27.8 ± 1.89	16.8 ± 2.19
1.0	20.5 ± 2.28	7.3 ± 1.93
0.5	11.6 ± 1.53	3.1 ± 1.23
0.25	6.5 ± 2.07	0.3 ± 0.2

	C	D
0.2	24.8 ± 3.31	21.6 ± 3.14
0.1	15.8 ± 1.54	14.0 ± 2.91
0.05	14.0 ± 2.91	7.5 ± 2.86
0.025	7.67 ± 1.96	6.0 ± 2.95

The data for Unacaine are recorded in Table 2. Solutions containing eserine show almost the same potency as those without (A and B). This may be explained on the probability that although eserine inhibits the hydrolysis of Unacaine it also increases its absorption. When adrenaline is added (C and D), the absorption factor is largely removed and the cholinesterase-inhibiting action of eserine becomes quite apparent.

Table 2

The anesthetic properties of Unacaine and additives. Values as in Table 1.

Solution A - Unacaine
 Solution B - Unacaine + eserine (25 $\mu\text{g/ml}$)
 Solution C - Unacaine + adrenaline (20 $\mu\text{g/ml}$)
 Solution D - Unacaine + eserine + adrenaline.

Concentration per cent.	Anesthetic potency	
	A	B
2.0	28.6 ± 0.9	29.5 ± 1.61
1.0	22.0 ± 1.97	21.5 ± 2.18
0.5	11.8 ± 2.58	16.0 ± 1.85
0.25	10.1 ± 1.36	8.5 ± 2.1
	C	D
0.2	28.5 ± 1.79	33.1 ± 1.2
0.1	16.3 ± 4.1	32.0 ± 1.3
0.05	5.3 ± 2.83	23.6 ± 2.3
0.025	1.67 ± 1.0	16.6 ± 4.45

Ravocaine (Table 3), follows very much the same pattern as does Procaine - a fact which our previously recorded studies would have led us to predict.

Discussion

The results of the guinea pig tests offer a confirmation of the enzymatic studies carried out previously in rabbits. At the same time they offer a convenient in vivo method of relating the anesthetic and metabolic characteristics of this group of drugs.

Table 3

The anesthetic properties of Ravocaine and Additives. Values as in Table 1.

Solution A	-	Ravocaine
Solution B	-	Ravocaine + eserine (25 µg/ml)
Solution C	-	Ravocaine + adrenaline (20 µg/ml)
Solution D	-	Ravocaine + eserine + adrenaline

Concentration per cent.	Anesthetic potency	
	A	B
1.0	26.8 ± 2.5	26.3 ± 1.3
0.5	24.5 ± 2.6	19.8 ± 2.7
0.25	15.2 ± 3.0	11.1 ± 0.8
0.125	10.3 ± 3.3	6.8 ± 1.3
	C	D
0.1	27.3 ± 2.4	29.1 ± 1.9
0.05	22.1 ± 1.9	25.6 ± 1.8
0.025	21.8 ± 2.3	22.0 ± 2.1
0.0125	13.3 ± 3.7	14.3 ± 4.3

These data lend weight to the thesis that s.c. toxicity of local anesthetics depends upon two factors, which may or may not be antagonistic. Thus a low s.c. toxicity may represent a poor diffusion (Procaine) or a rapid local hydrolysis (Unacaine). Conversely a high s.c. toxicity may reflect rapid diffusion and/or a stability toward cholinesterase. Of the two factors - diffusion and hydrolysis - the latter is the more important in determining systemic toxicity; for if a drug diffuses slowly from the site of deposition into the blood, the concentration in the blood at any given time will be low and the chances of toxic action small. On the other hand, if a drug enters the blood rapidly, then the changes of a toxic action manifesting itself will depend upon how rapidly it is destroyed by enzymatic hydrolysis.

A determination of enzymatic hydrolysis would seem, therefore, to be important in reporting data on the toxicity of local anesthetics. This is most conveniently carried out by methods which have been previously described, namely by chemical determination of the rate of disappearance of the drug after it has been mixed with rabbit blood and incubated at 37°C.

One further point in connection with the guinea pig tests should be noted. It is doubtful whether eserine should be used clinically for its presence in the blood would cause a general inhibition of pseudo-cholinesterase thereby removing the body's most important mechanism of defence against the toxic action of the drug. Furthermore, eserine would exhibit certain undesirable side reactions also by virtue of its cholinesterase-inhibiting properties.

Section B - The metabolism of procaine hydrochloride and related compounds. III. Oxycaïne hydrochloride (diethylaminoethyl-4-amino salicylic acid)

This drug was subjected to the various biological and chemical tests which had been applied in the study of Unacaine and Ravocaine. The following findings were made:

- (a) The maximum rate of metabolism in the rabbit is about 1.1 mg/kg/min. (Procaine was found to have a value of 1.5). Data from the manufacturer indicates that the i.v. convulsing dose in rats is 11 mg/kg, whereas that for Procaine is 15 mg/kg.
- (b) Oxycaïne is very stable toward pseudo-cholinesterase, no detectable breakdown occurring after 30 minutes incubation in rabbit blood at 37°C.
- (c) Pre-convulsing blood concentrations showed the absence of a prominent blood-brain concentration gradient. This would indicate a fairly rapid rate of diffusion.

In summary, this compound is similar to Procaine in some respects except that it is much less readily metabolized. Although the latter characteristic makes for a longer duration of action, it also increases toxicity. Indirect evidence suggests that the rate of diffusion of Oxycaïne from blood to brain may be somewhat greater than that of Procaine.

IV. Primacaine hydrochloride (diethylamino ethyl-2-butoxy-3-amino benzoic acid).

Considerable difficulty has been experienced in adapting

the method of Terp to the quantitative determination of this drug. Recently progress has been made in overcoming these difficulties, and it is therefore hoped that this stage of the project will be completed during 1957-58.

Section B - The metabolism of procaine hydrochloride and related compounds. III. Oxyacine hydrochloride (diethylaminoethyl-4-amino salicylic acid)

This drug was subjected to the various biological and chemical tests which had been applied in the study of Procaine and Novocaine. The following findings were made:

- (a) The maximum rate of metabolism in the rabbit is about 1.1 mg/kg/min. (Procaine was found to have a value of 1.2). Data from the manufacturer indicates that the rate of metabolism of Procaine is 1.1 mg/kg, whereas that for Procaine is 1.2 mg/kg.
- (b) Oxyacine is very stable toward pseudo-cholinesterase, no detectable breakdown occurring after 30 minutes incubation in rabbit blood at 37°C.
- (c) Pre-convulsant blood concentrations showed the absence of a pre-convulsant blood concentration gradient. However, a pre-convulsant blood concentration gradient was observed in the case of Procaine. In the case of Procaine, the blood concentration was found to be higher in the blood of the animal which was convulsed than in the blood of the animal which was not convulsed. This suggests that the rate of elimination of Procaine from the blood is slower than that of Oxyacine. In the case of Oxyacine, the blood concentration was found to be lower in the blood of the animal which was convulsed than in the blood of the animal which was not convulsed. This suggests that the rate of elimination of Oxyacine from the blood is faster than that of Procaine.

APPENDIX D

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: Studies on the mechanism of gingival changes associated with drug administration

Grantee: Dr. K. I. Melville,
Dept. of Pharmacology, McGill University

Project No.: D.R. 66 Amount of Grant: \$3,000.

During the first six months, various types of exploratory and preliminary experiments were performed in order to establish a convenient and reliable procedure for the production of experimental gingival hyperplasia. Both young and adult rats, hamsters, guinea pigs and ferrets were kept under as far as possible uniform conditions of diet (either ordinary laboratory chow or a laboratory synthetic diet was used). Small groups of 4 to 6 animals of each species were given increasing doses (5 to 40 mgm. daily) of diphenylhydantoin either incorporated in the diet or by stomach tube.

In some experiments the drug was employed as the alkaline sodium salt in solution and injected repeatedly locally under aseptic conditions into the muco-buccal fold. In comparison with similar untreated control groups, animals maintained for as long as 3 months, with weekly examinations of the gums, as well as observations of their general physical state (body weight, etc.) of the animals, revealed no gross or specific microscopic abnormal changes in the gums, even at the sites of local injections.

On the other hand, with more prolonged (6 to 8 months) oral administrations of the drug, while no gross gingival changes were noted in rats, some microscopic hyperplasia of the epithelial and connective tissue of the gums was observed in some animals, but not in others. However, with similar prolonged administrations to adult ferrets it was observed that three animals which survived 32 weeks developed reddening and swelling of the gums with definite overgrowth of the gingival folds tending to envelop the teeth in some areas. These animals are still under observation, and microscopic tissue studies will be carried out in due course. In any event these observations on ferrets appear to confirm those of King in England, but leave no doubt that the method is still rather unsatisfactory, in view of the prolonged (8 months or longer) periods required to achieve the gingival

changes. It is however clear that the ferret is the most suitable animal for the production of this type of experimental gum hyperplasia, and with the hope of inducing similar gingival changes more rapidly, two new series of ferrets have already been started, using higher daily dose levels (40 to 100 mgm.), and employing both young (8 weeks old) and adult animals.

Although hyperplasia in tissues other than the gums, has not been reported in man, there was some suggestion of thickening of the skin in some of the rats subjected to prolonged treatment as indicated above. Moreover, it has been suggested that gingival hyperplasia following diphenylhydantoin therapy might be a "hypersensitivity or allergenic reaction". It was therefore of interest to study the histamine and serotonin contents of the gingiva of both normal and diphenylhydantoin-treated animals. Experiments along these lines using suitable biological assay methods have also be initiated. So far as we are aware, there are no published studies in the literature concerning the histamine and serotonin contents of the gum, and from our preliminary assays it appears that the gingiva is perhaps as rich as the intestinal mucosa in histamine, and apart from its possible role in the problem of hyperplasia, further studies along these lines are highly desirable.

Finally, using strips of isolated guinea pigs' ileum, suspended in a bath and recording responses to acetylcholine, histamine and serotonin, the effects of diphenylhydantoin and N-methylphenylethylhydantoin (mesantoin) were compared. It has so far been observed that diphenylhydantoin exerts potent anti-acetylcholine action, some antihistamine and less anti-serotonin actions but these effects could not be observed with the latter agent. Whether or not they are related to the fact that gingival hyperplasia occurs with the one but not with the other agent requires further investigation.

In conclusion, the results of these various preliminary studies, which were initiated during the year, appear to be sufficiently encouraging to warrant continuation and expansion of research in this obscure area of dental interest, and it is hoped that funds will be available to do so.

APPENDIX E

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: A study of developing human intradental and peridental vascular patterns by micro-radiographic methods.

Grantee: Dr. R. L. deC. H. Saunders,
Dept. of Anatomy, Dalhousie University

Project No.: D. R. 51 **Amount of Grant:** \$2,130.

Fresh human fetuses immediately after delivery were placed in a saline bath maintained at body temperature. Retrograde aortic injection was then carried out by intubating the descending thoracic aorta with fine bore polyethylene tubing. A fine grain radiopaque injectant consisting of Micropaque (25%) made up in sodium citrate (3.8%) was used, since the average particle size of this radiopaque solution is many times smaller (0.5 micron; checked by electron microscope) than capillary diameter, and consequently it readily penetrates all parts of the vascular network. Owing to the low range of foetal blood pressure (e.g. 55/25 mm.Hg. at 26 weeks) special attention was paid to injection pressures. The foetal jaws were then excised and microradiographed to determine the state of injection of the foetal jaw and contained dental germs, following which the gingival tissues were incised to expose the subjacent dental sacs and contained dental germs. These last were then removed under a dissecting microscope for microradiographic study. Contact microradiographs were made by placing the specimens on contrast slides or maximum resolution plates, the exposure factors naturally varying with the specimen and focal spot of the tube employed.

The microradiography equipment used was a Phillips Norelco watercooled diffraction tube with a spot focus of 1.0 x 1.2 mm., equipped with beryllium windows and a copper target. A Hilger Microfocus (.04 mm) demountable tube with interchangeable targets of copper, chromium, etc., has been used more recently. This has given sharper images especially when used with a vacuum pumped camera, and allowed of some degree of direct magnification, i.e. projection microradiography.

The material studied to date has consisted of foetal jaws obtained from 45 fresh human foetuses of various ages ranging between the 3rd and 6th month. Microradiographic appearances have been confirmed both by means of the dissecting microscope and histological sections. Between 900 and 1000 slides of transverse and longitudinal sections of the foetal jaw have been made.

The microradiographic studies of the dental pulp vessels of the human adult carried out over the past four years have been continued. During the past year forty adult teeth have been injected with contrast medium, using the suction-injection technique, and studied microradiographically. Comparison studies of injected and non-injected human dental pulps using microradiographic and histological methods were carried out. Transverse sections of injected and non-injected pulp were microradiographed successfully, and further experimentation in this direction is contemplated. Serial histological cross sections of the tooth pulp of various teeth supported and confirmed the vascular pattern of the pulp as imaged microradiographically.

Results

These microradiographic methods have provided the first complete pictures of the vascular networks lying within the dental papilla and about the dental sac of the developing human tooth. Two vascular nets or plexuses were demonstrated lying in relation to the developing teeth within the foetal jaw. One plexus appeared to lie about the dental sac (dental follicle), or embryonic capsule surrounding the developing tooth, and hence was named the peridental (perifollicular) vascular plexus. Histological sections showed that this plexus was closely related to the outermost layer of the enamel organ. Another plexus lay below the developing crown within the dental papilla or precursor of the dental pulp, and so it was named the intradental (intrapapillary) vascular plexus. The position of these vascular plexuses was confirmed both under the dissecting microscope as well as histologically.

An interesting feature of some importance is the fact that the capillary network of the intradental plexus evidently precedes the appearance of the enamel and mineralization of the crown. This was suggested by the marked vascularization of the dental papilla, which was easily seen through the uncalcified organic matrix of the crown,

while the dental cusps were just beginning to show signs of maturation and calcification as evidenced by increasing radiopacity at their tips (see fig. 9 of exhibit). Large subcuspal vessels are early selected from the intradental plexus to become the main pulpal arteries and veins, and herald the future principal root vessels. The final general pattern assumed by the intradental plexus was similar in single and multirooted teeth, although in molars, parts of this plexus apparently persist forming a vascular bridge across the pulp chamber between the root canal vessels (see fig. 11 of exhibit).

Microradiographs of injected adult teeth showed that the apical arteries on entering the root gave rise to a bundle of central or principal pulpal arteries which ascended the root canal and pulp chamber, the smaller calibre and straighter course of the arteries distinguishing them from the wider beaded silhouette of the pulpal veins. The central arteries were surrounded by intermediate sized paraxial vessels from which numerous small branches passed radially to terminate in the peripheral (subdental) plexus. The peripheral (subdental) plexus was found to consist not only of capillary loops, but was actually an anastomotic network lying near the pulp surface that extended from the upper (coronal) part of the pulp chamber well down into the root canal (see fig. 14 of exhibit) and presenting an irregular subdental contour (see figs. 12, 13, 14 of exhibit). This peripheral or subdental capillary plexus drained via wide subjacent venules which then passed centrally to join the larger tributaries of what have here been named the major pulpal veins. These major veins underwent a reduction in calibre during their descent, while their tributaries often joined them with a terminal constriction.

Comments

The intradental and peridental vascular plexuses found in the human foetus, must be related to (i) the developmental production of dentine and enamel, (ii) the processes attending the mineralization and eruption of the tooth, and (iii) apparently provide the means whereby calcium and other salts, as well as fluorides, are conveyed to the developing tooth. Also (iv) prenatal infections (e.g. syphilis, rubella) must similarly gain the tooth germ via these plexuses, thereby explaining the deformed Hutchinsonian teeth of congenital syphilis, and certain types of dental hypoplasia.

Certain workers have stated that the dental pulp vessels were too small to be followed on radiographs. The microradiographic methods here used have demonstrated the intact vascular patterns of the developing and adult tooth in their entirety, and the pulpal vessels imaged down to the finest capillaries of the peripheral or subdental plexus.

The microradiographic studies carried out under this grant (D.R. 51) were reported before the First International Symposium on Microradiography and X-Ray Microscopy recently held at Cambridge, and are being published in the Proceedings, which are now in press. Another account is appearing in Mr. R.V. Ely's (Research Director, Lancashire Electric etc.) forthcoming book on Microradiography and X-Ray Microscopy, which is also in press. A further account of the work carried out under this grant was presented at the Maritime Dental Convention held in Halifax last July.

A continuance of this grant (D.R. 51) has not been requested at present since the writer has accepted an invitation to work at the Cavendish Laboratory, Cambridge University, on the Cosslett & Nixon X-Ray Shadow Microscope, where he is at present spending a year developing vascular techniques required in using this instrument. The instrument possesses an electron gun with two magnetic reducing lenses whereby a point source of electrons is produced, which on striking a thin window anode or target at the end of the tube, produces an X-ray source of less than 1 micron in size at 10 kv. Experiments in projection microradiography or X-ray microscopy are being carried out, a large initial magnification being secured by X-ray projection and sharp images obtained from thick specimens opaque to the ordinary optical microscope, with marked improvement in resolution. It can already be stated that dental studies made with this instrument are superior to those obtained by contact microradiography, as may be judged from the X-ray micrograph of dental pulp showing the capillaries of the human peripheral (subdental) plexus imaged in juxtaposition to a piece of 1500/inch silver grid composed of 3 micron bars (see exhibit fig A) and the X-ray micrographs forwarded to the Advisory Committee on Medical Research.

An improved model (Mark 11) of this X-ray microscope has been built for the writer by Mr. R. Ely with Dr. V.E. Cosslett and Dr. W. Nixon acting in a professional advisory capacity, and is now being tested in the Cavendish with their assistance. A twin instrument was built at the same time for the Royal Caroline (Nobel) Institute, Stockholm.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

These instruments possess a magnification range of x10 to x1000, and resolution of 0.1 micron as opposed to the best American effort of 1 micron.

The writer has applied to the National Research Council of Canada for a non-recurring equipment grant for 1956-57 to the amount of \$5,000 to cover the cost of manufacture, installation and freight of this instrument. (It might interest the Committee to know that the General Electric Co. of America have quoted the sum of \$10,500 for an inferior instrument equipped with electrostatic lenses and a more limited kilovoltage range, to which must be added 5% for export and freight.) On returning to Canada the writer intends to continue his research in the dental field extending the work now in progress at the Cavendish Laboratory.

The resignation of Dr. Jenkins from the staff of the University of Toronto in June 1955, along with the elimination of the research requirement from the curriculum for the future candidates for the Diploma in Orthodontics has made it necessary to forego the extension of this work for the foreseeable future. Nevertheless, the results of the project to date have been very substantial, and are embodied in the following Master of Science theses on file at the Faculty of Dentistry, University of Toronto. The total of the written reports runs to close to 300 pages.

1. Study of the Dentofacial Morphology and Temporalis Muscle Activity in Normal Seven-Year-Old Children employing Electromyography and Cephalometric Radiography. E.B. Cook, filed May 1954.
2. Study of Dento-facial Asymmetry, Dental Occlusion and Temporal Muscle Activity in Normal Eight-Year-Old Children. E.J. Levin, filed May 1955.
3. Study of Dentofacial Morphology and Temporalis Muscle Activity in Normal Seven-Year-Old Children, Employing Cephalometric Radiographs and Electromyography. G.W. Street, filed May 1954.
4. An Evaluation of the Techniques for Electromyography of the Temporal Muscle and a Study of Certain Aspects of its Function in a Group of Children Exhibiting Distocclusion. R. Boyko, filed May 1956.
5. An Electromyographic and Cephalographic Study of Normal Five- to Eight-Year-Old Children. D.S. Woodson, filed November 1956.

APPENDIX F

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: Continuing studies in electromyographic techniques

Grantee: Dr. D. H. Jenkins

Project No.: D.R. 37 Amount of Grant: nil

This project has been carried on by Dr. H. Jenkins while Head of the Department of Orthodontics, University of Toronto, with the active work being done by graduate students. The resignation of Dr. Jenkins from the staff of the University of Toronto in June 1956, along with the elimination of the research requirement from the curriculum for the future candidates for the Diploma in Orthodontics has made it necessary to forego the extension of this work for the foreseeable future. Nevertheless, the results of the project to date have been very substantial, and are embodied in the following Master of Science theses on file at the Faculty of Dentistry, University of Toronto. The total of the written reports runs to close to 300 pages.

1. Study of the Dentofacial Morphology and Temporalis Muscle Activity in Normal Seven-Year-Old Children employing Electromyography and Cephalometric Radiography. E.B. Cook, filed May 1954.
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3. Study of Dentofacial Morphology and Temporalis Muscle Activity in Normal Seven-Year-Old Children, Employing Cephalometric Radiographs and Electromyography. G.W. Street, filed May 1954.
4. An Evaluation of the Techniques for Electromyography of the Temporal Muscle and a Study of Certain Aspects of Its Function in a Group of Children Exhibiting Distocclusion. R. Boyko, filed May 1956.
5. An Electromyographic and Cephalographic Study of Normal Five- to Eight-Year-Old Children. D.G. Woodside, filed November 1956.

6. An Electromyographic Study of the Postural Activity of the Temporal Muscle and Its Relation to Mandibular Rest Position. M.C. Johnston, filed November 1956.

The major conclusions are found in the latter papers which of course build on the knowledge of the previous workers. One further paper by D. Allen is to be completed by May, 1957. The following paragraphs from the work of D. Woodside and M. Johnston effectively summarize the present status of the work:

Woodside, middle of page 78

"It was concluded that the activity pattern of the posterior temporal muscle in young children with either normal occlusion in the mixed dentition stage shows wide variation, and that the differences in posterior temporal muscle activity between children with Class II type occlusion and those maintaining normal occlusion in the mixed dentition stage were not significant. Because of the small sample these latter findings might be compatible with the existence of a real difference. It was conjectured that the activity of the muscle is closely related to and caused by occlusal interference. It was concluded that while the broad activity classification of the individual child in the mixed dentition stage could be expected to remain the same during a yearly interval, the detailed picture of activity in the individual was very variable during the yearly interval. When changes in broad activity classification do occur, they need not be accompanied by easily discernable gross occlusal changes although they may be accompanied by minor changes."

Johnston, pages 71 and 72

"Possible value of electromyography in dentistry: The contribution of electromyographic studies to background knowledge relating to etiology, possibilities of treatment and prognosis would appear to be of considerable value.

"Direct clinical application of electromyography does not seem to be practical as yet at least. However, the work being conducted by Perry in this respect is interesting. He found that, by using two large surface recording electrodes (one over the masseter, and one over the temporal), a high degree of reproducibility in reset position cephalometric radiographs could be attained by insuring a minimum of activity in the temporal mandibular musculature as observed in simultaneous (with radiographic exposure) electromyographic recordings."

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In the latter papers, much time has been spent at the formidable but logical task of determining the physiological meaning of the recordings being produced by the tracing pens and of the ways and means of interpreting the recordings quantitatively. M. Johnston produced a quick method of estimating quantitatively the physiologic activity reflected by recordings of specific amplitude and frequency. The current work of D. Allen is to test the effectiveness of Johnston's comparison scale against known temporal muscle stress produced by hanging weights and to compare the system of paper recording with recording by automatic counters and by photographs of oscilloscope pictures.

It is not impossible that further work may be undertaken with this problem probably in conjunction with the major Orthodontic Research Project in Burlington, Ontario, which is sponsored by a National Health Grant.

Further light on the etiology, treatment and prevention of periodontal disease. However, it has been found that some of the findings may have application in fields other than periodontia, in both dentistry and medicine. In this report dealing with the more general aspects of the work, the etiology, physiology, pathology rather than the application of the research to one specific field.

Earlier studies in this investigation have established (experimentally in dogs) (1) that gingival tissue detached from a tooth may be induced to reattach (Study 1); (2) that in addition to soft tissue reattachment, lost bone of the socket and periodontal membrane may be regenerated (Study 2); (3) Gingival tissue detached during the course of periodontal disease is covered by the apical proliferation of the oral epithelium. This action of the oral epithelium plus the loss of socket bone and periodontal membrane has the effect of taking the denuded area of the tooth from the internal to the external environment. It was important to establish whether or not the regeneration of supporting structure could be induced to occur in this situation. It was found that regeneration of the lost structure from epithelialized sockets could occur in two ways: (a) following surgical interference (Study 4), and (b) apparently spontaneously over a period of time (Study 3).

Having established experimentally that regeneration of lost supporting structure is biologically possible, a further objective was the clinical application. For many reasons this phase of the investigations is difficult and complex.

APPENDIX G

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: An investigation of the mechanism of repair of the supporting structures of the teeth.

Grantee: Dr. C.H. Best (Dr. W.J. Linghorne)

Project No.: D.R. 22 Amount of Grant: \$3,975.00

Summary of Work

One objective of this investigation is the development of a more rational approach to problems in dental practice involving the supporting structures of the teeth. Particular emphasis has been given to the research that may throw further light on the etiology, treatment and prevention of periodontal disease. However, it has been found that some of the findings may have application in fields other than periodontia, in both dentistry and medicine. So this report deals with the more general aspects of the work, the basic histology, physiology, pathology rather than the application of the research to one specific field.

Earlier studies in this investigation have established (experimentally in dogs) (1) that gingival tissue detached from a tooth may be induced to reattach (Study 1); (2) that in addition to soft tissue reattachment, lost bone of the socket and periodontal membrane may be regenerated (Study 2); (3) Gingival tissue detached during the course of periodontal disease is covered by the apical proliferation of the oral epithelium. This action of the oral epithelium plus the loss of socket bone and periodontal membrane has the effect of taking the denuded area of the tooth from the internal to the external environment. It was important to establish whether or not the regeneration of supporting structure could be induced to occur in this situation. It was found that regeneration of the lost structure from epithelialized pockets could occur in two ways: (a) following surgical interference (Study 4), and (b) apparently spontaneously over a period of time (Study 3).

Having established experimentally that regeneration of lost supporting structure is biologically possible, a further objective was the clinical application. For many reasons this phase of the investigations is difficult and complex.

The etiology of periodontal disease is obscure. In addition there are difficulties imposed by the nature of the problem and by the limitations of dental practice.

In considering the clarification of the etiology of periodontal disease the question arises: Why are so many people unable to maintain the integrity of the part of the skeletal system concerned with the support of the teeth? An understanding of the manner in which skeletal tissues are maintained would be very useful in understanding the failure in the maintenance of one part. Similarly a rational approach to the repair of damage to the skeletal tissues supporting the teeth would be aided by an understanding of the manner in which the skeletal tissues of the body are repaired. Thus our investigation has been directed toward a study of the mechanisms concerned with the maintenance and repair of the skeletal system.

A seminar reporting the progress in this phase of our investigation is being prepared for the consideration and criticism of Dr. Best, members of the staff at the Institute, the Dean and members of the staff of the Faculty of Dentistry, members of the Associate Committee on Dental Research, and others interested in this field. As this report gives a review of the work in this investigation, its present status and the lines along which we hope to proceed, it is appended as an interim report.

Progress Report

My subject is "The Maintenance and Repair of the Skeletal Tissues." My interest in this subject is a result of my research program, one of the main objectives of which is the development of a more rational approach to the treatment and control of a type of disease affecting the masticatory apparatus. The masticatory apparatus, like the arm or leg, is a biologic mechanical function having skeletal support. The part of the skeletal support of the masticatory apparatus I am most interested in, is that part of the jaws concerned with the support of the teeth. Disease affecting these tissues is called periodontal disease.

During the course of periodontal disease the teeth gradually become detached from their support and that part of the bone of the jaw forming the sockets of the teeth is gradually destroyed. The etiology of this type of disease is obscure and for that reason treatment of this disease has been developed largely empirically and is to a great extent palliative in nature. When considering what is required for a more rational approach to periodontal disease, two basic problems become apparent. 1) In the environment of to-day why is such a large proportion of people unable to maintain this part of the skeletal system? Now, it is unlikely that the basic maintenance procedures differ in the various parts of the skeletal system. If we understood how the skeletal tissues of the body are maintained we would have gone far toward understanding the failure of maintenance of one part.

The next problem is, how can the damaged structures be repaired? Again, if we understood the mechanism by which skeletal tissues are repaired, we would have a basis on which to develop a more rational approach to this phase of our clinical problem.

Kelgren in the Gualstonian Lecture in 1952 stated that "the mechanisms by which the supporting tissues of the body are produced and maintained is unknown". In a sense that is true, but research on the skeletal tissues has been going on for many years and a large amount of data has been developed. In addition to the experimental data, there is a voluminous literature, especially on bone, from the clinical side. But surgeons still have problems in healing, especially with certain types of skeletal lesions and diseased conditions. Also there is definitely not enough known about the mechanisms concerned in maintenance and repair to help much in our problem of periodontal disease.

One of the reasons for this situation is that bone, the main skeletal tissue, is a difficult tissue to study. Like other supporting tissues of the body, bone is composed of living cells and nonliving intercellular substance. Fundamentally the main difference between bone and other supporting tissues is that in bone the nonliving intercellular substance becomes calcified. This does not seem to be so very important but in a recent paper Dr. Ham pointed out that calcification imposes peculiar difficulties on the nutrition, repair and growth and grafting of bone when compared with uncalcified tissues. It can be assumed, then, that the maintenance of bone is more complex. Another difference between supporting tissues and other tissues such as glandular, nerve or muscle tissue, is that one of their most important functions, support, is carried out not by living cells, but by the nonliving intercellular substance.

In preparing this paper there had to be limitations, so my discussion will be largely confined to bone and connective tissue. There is a voluminous literature on these tissues as separate tissues. I wish to deal with them from a limited point of view, as they are integrated to form the skeletal system. I will thus be discussing only their supportive function. Again, in the literature, growth, maintenance and repair often are linked together. I will have little to say about growth. As a further limitation discussion of another skeletal tissue, cartilage, will be largely omitted.

Slide 1. This slide of the skeletal tissues of the foot illustrates this approach to connective tissues and bone as they are integrated to form the skeletal system. We see the skeletal system consists of a number of bones fastened together by connective tissue forming joints. We are accustomed to consider the main reason for this type of arrangement is to permit movement. But as Dr. Ham states "the permitting of movement is not essential for a connective structure to be termed a joint". He defines joints as those structural arrangements that exist to connect two or more bones together at the site of their meeting. Some joints are freely moveable, others are almost as solid as the bones they connect. The degree of movement in joints varies between these two extremes. In some of the moving joints the articulating surfaces are covered with cartilage. These are called synchondroses. In others called synovial joints, there is a cavity containing fluid for lubrication. But as I am limiting our discussion to bone and connective tissue as they form the jointed skeletal system, the joints I shall discuss are syndemoses. Ham defined syndemoses as joints wherein bones at their site of meeting are held together by

bands of dense fibrous tissue. He adds further that it is important to appreciate that in a syndemosis, the bands of connective tissue extend from one bare bony surface to another.

Slide 2. In the next slide we see that the connection between the teeth and the bone of the jaws may properly be termed joints of the syndemosis type. Perhaps we might change our perspective; instead of thinking of joints as primarily for movement, let us consider that the various calcified parts of the skeletal system must be formed separately. These separately formed parts must then be fastened together to meet the requirements of skeletal support. Instead of thinking of movement as the primary purpose of a jointed skeleton, movement then becomes an adaptation. We then have no trouble in thinking of the attachment of the teeth to the jaws as joints. We can think of teeth and jaws as parts of the skeletal support of the masticatory apparatus formed separately and then joined together. This is done by connective tissue. Also we see that periodontal disease is then a type of joint disease.

Now, it is unlikely that the basic physiological mechanisms in maintenance and repair differ in joints in the various parts of the body. Therefore studies of the physiology of one joint should apply to other joints, with certain adjustments to the local situation. Also a study of disease affecting one joint may throw some light on other joint diseases. So when I speak of the attachment of the tooth to the jaw, I will use the term "dental joint" to distinguish it from other joints in the body. I hope the clinical people present will think about what I have to say in terms of the joints in which they are most interested.

This might be a suitable time to point out one of the local features in which the joint between the tooth and the jaw differs from other joints in the body. (Slide of tooth) We see that the dental joint is bounded at one end by epithelium. Thus when through trauma or disease the connection between the tooth and the bone is severed, the epithelium proliferates and covers the severed tissues. This has the effect of taking the damaged part of the joint from the internal to the external environment. Once this has occurred, the reconstruction of the damaged articulation can not occur, at least in the usual manner. This action of the epithelium complicates dental therapeutics but it has been quite useful when studying regenerative procedures as we will see later.

In order to get the general picture I will briefly review my earlier investigations. One of the first objectives was to ascertain if regeneration of normal dental joint could be induced if part of it was not just detached or severed, but completely destroyed, by the removal of the socket bone,

connective tissue and the cementum. Slide 5. The next slide shows a regenerated section of the joint. For the present I shall not discuss the methods used to induce this regeneration, but rather some of the questions the attainment of this result brings up. In the section of the joint that was destroyed, everything was removed: bone, cementum and connective tissue. To regenerate the joint all three tissues had to be formed in this area. What type of cell or cells were needed for this regeneration? Obviously we needed osteoblasts, cementoblasts and fibroblasts. Where did they come from and what caused their specialized development in the correct location to produce this regeneration? Is there a special mechanism to form cementum, another to form connective tissue and still another to form bone? In the next few slides being shown, is some of the most direct evidence I have that all three tissues can be formed from the same type of cell. Slide 6. First cementum and bone. This slide is of a case at 30 days when regeneration of the bone and cementum is taking place. Grafts were used in this case and on this side we see new bone being formed. This will eventually be bone, this is the fibrous connective tissue and this the new cementum. Please decide if this new bone and new cementum are one and the same tissue.

Now the evidence suggests that bone and cementum are formed by the same type of cell. How about the connective tissue? Slide 7. This is another case where regeneration was induced. Notice in this slide we see the usual regeneration of bone, connective tissue and cementum forming the joint. Slide 8. The next slide is a little further into the block. Notice here that the differentiation to connective tissue has not taken place. Here it is bone marrow. Slide 9. Still deeper into the block we see that we have an ankylosed joint. The bone, cementum and tooth are solid. You can imagine how the ankylosis restricted movement in the joint. This restriction of movement may possibly be one reason for the lack of differentiation to form connective tissue. In any case, it does seem that the connective tissue of the joint differentiated from the same cells as does bone marrow. From this and other evidence which we shall see later, part of our concept is that in the repair of any defect in the skeletal system proliferating osteogenic cells have the genetic potency to form either bone, cementum, connective tissue or cartilage. Later I hope to discuss some of the factors concerned in determining the type of mature tissue formed in the healing of a defect.

Now you remember I mentioned a factor peculiar to the dental joint, the presence of epithelium bounding one end. In periodontal disease as the connection between the teeth

and the supporting structure is severed, the epithelium proliferates over the detached tissue toward the apex of the tooth. The action of the epithelium takes the damaged joint from the internal to the external environment. Slide 10. It was important from the dental point of view to learn whether or not the lost socket bone and the damaged part of the joint could be regenerated, under these conditions. A provisional hypothesis of the requirement for this regeneration was worked out from the previous studies and applied to this problem. Slide 11. Here is the regenerated joint.

Later in this talk the principles underlying the methods used to regenerate the joint will be discussed. I feel it will be more suitable then. I mention this phase of the work now, as not until the studies with the regeneration of the dental joint with the epithelial complication did I realize that the regeneration of a joint could occur in two ways: 1) following surgical interference, and 2) apparently spontaneously over a period of time, one to three years.

So far we have been talking about the regeneration of the dental joint following surgical interference. For many reasons, the finding of the second method, the spontaneous slow regeneration of lost joint structure without surgical intervention, was more exciting from the dental viewpoint. For reasons with which I shall not bore you, the study of this spontaneous regeneration does not lend itself to animal experimentation, at least not with the facilities at my disposal. But if we could learn sufficient about this regenerative method to be able to initiate it clinically, it would be a milestone in the treatment of periodontal disease. Also, the etiology of periodontal disease is obscure. I felt that if we understood the nature of the spontaneous regeneration mechanism, much would be learned about the etiology of this disease.

After struggling with this problem of spontaneous regeneration and finding no direct method of approach to it, I wondered if it could be approached indirectly. Let me explain. So far in my work I built up a fair collection of histological sections of skeletal tissue repaired or being repaired under a variety of experimental conditions. In addition, research of the skeletal tissues has been going on for a long time. There was a good chance that there was already sufficient experimental data for the solution of my problem if I could interpret it. And here is where my

troubles began. I wanted to apply an experimental result clinically; one feels on safe ground when reporting a new fact that is confirmable. I felt that some different procedure is necessary to be able to apply scientific data clinically, but I was not clear what this procedure was and how to go about it. I was struck by the similarity between my problem and that described by Sir Howard Flores in his concluding remarks in his lecture on the present status of atherosclerosis. He said: "There is no lack of experimental data on this subject. The problem is, what is relevant?" Obviously some further step must be taken before the clinician treating or preventing atherosclerosis can make full use of the scientific data available. But what is the step? Among other things I turned to the literature on the philosophy of science, scientific logic and methods, etc. I enjoyed the description given by one chap regarding such a situation, as propounded by Sir Howard Flores. He described it as "confused, complex and disorderly even though endowed with direct confirmability". That is how I felt about the large amount of data in the bone and connective tissue field. In such a situation, I found that the development of a concept or theory on the basis of the confirmable facts was a recognized scientific procedure. However there was quite a difference of opinion about the procedure itself. Duhem described such a theory as "merely an instrument for ordering and classifying experience"; Mack as a "collection of as many facts as possible in a synoptical form" and Pierson called a scientific theory "mental shorthand". Black disagrees with the view that a theory is a device for the summarization of facts. He points out that "one of the most striking things about a theory is its predictive value, its value to the investigator is not only to tie up bundles of facts already in his possession but it leads to the promulgation and confirmation of laws which could never have been detected without its help". Each of these views presents a different phase of the usefulness in this step. Thus we see that at a certain stage in an investigation such a procedure seems to be a way or device to organize, consolidate and utilize present gains, and at the same time facilitate further progress. But we must recognize that future findings investigated as a result of the theory may modify or change the theory. This still does not detract from the value of this procedure. Only gradually and at this stage did it become clear that I needed a concept of the manner by which the skeletal tissues are (1) maintained and (2) repaired.

To further clarify my objectives let me state one objective another way. I have often asked clinicians why they treat a case in a certain way. Generally they give the rationale for their treatment, that is the theoretical basis on which the treatment is planned. Whether or not we are conscious of it, most of the time there is a rationale underlying treatment. One objective of this work is to improve the rationale underlying treatment of disturbances of the skeletal tissues. As would be expected, then, this paper has a clinical slant. I am reporting the progress made in this phase of my investigation for your consideration and criticism.

My plan is to present the relevant data from my own work and that of others, with my interpretation. Time will not permit a discussion of all the studies that tend to support or contradict my interpretation, but I will mention one or two with each step. Then I shall use the concept as a tool in considering some common clinical problems about which much is known. One reason for so doing is to obtain the criticism of men in the different fields who are dealing with the skeletal tissues in their daily work. So let us begin.

In the healing of a defect, bone is not simply patched together by scar tissue, as in the healing of other organs. Bone repair is ordinarily so complete that it is impossible to find in a histological section the area of a fracture or large defect one year after injury. Let us see if this fact is also true in joint regeneration. Slide 12. The first slide is regeneration following surgical intervention. Slide 13. The second following surgical intervention after the epithelium is allowed to proliferate over the detached tissue. Slide 14. Here the same conditions prevailed but healing occurred by the method I refer to as spontaneous. We can see no clue as to the method by which regeneration occurred. In other words, I could substitute any of the slides and no one could tell the difference after healing is completed. Except for the resorption of the dentine of the tooth, there would be no clue as to whether or not the section was in the area of the defect. The reason the resorption of the tooth provides permanent evidence of the defect is that the dentine is a non-vascular calcified tissue. You can appreciate the importance from the research viewpoint of being able to find the defect in a histological section. This is one advantage of using dental tissues. So we see that ultimately, if regeneration of a joint does occur, the end result is the same regardless of the type of lesion or the method by which the healing took place.

Now let us compare the differences in the healing processes before healing is completed. It might be helpful if I first refresh your memories about normal bone. These next few slides of bone are taken from text books. Slide 15. This slide is a cross-section through a Haversian system, compact bone. We know that bone consists of a collagenous matrix of fibers, which are embedded in a homogenous ground substance. The whole is rendered rigid by deposition of calcium salts. We see that in an Haversian system bone is arranged as a number of concentric lamellae which are grouped around a narrow axial canal. The regular arrangement of the fibrils is associated with a regular arrangement and orientation of the bone cells and the cell spaces, the lacunae. The cells and cell spaces tend to be elongated in the direction of the fibrils. Slide 16. In the next slide showing cancellous bone, we also see the same regular arrangement of the fibrils, and osteocytes forming lamellae. I wish to draw your attention to the fact that in both compact and cancellous bone the bone is laid down in lamellae with this regular arrangement of the fibrils and cells. Slide 17. In the next slide, stained with silver impregnation, we see the reason for the lamellar appearance. The fibrils are arranged in sheets; in each sheet the general direction of the fibrils is the same but on passing to the next sheet of fibrils the general direction of the fibrils changes, as a rule through an angle of about 45° , though sometimes through a complete right angle. This alteration in the direction of the fibrils produces the lamellar appearance. The lamellar appearance is most distinctive where the change of angle is greatest. The effect of this plywood-like arrangement is to increase strength against shearing. Thus we see that compact and cancellous bone is characterized by a certain pattern in the arrangement of the bone cells and the cell spaces. Some authors use the terms lamellar or lamellated of bone formed with this rather complicated structure.

Now let us look at the bone formed in the regeneration following surgical interference. Slide 18. This bone is laid down in trabeculae, and for that reason it is often called cancellous bone. But is it cancellous bone? We have seen that cancellous bone shows the characteristic pattern of the fibrils and cells of lamellar bone. In this bone the fibrils of the matrix cross one another in different directions forming an irregular woven felt work. Notice the different shape and lack of orientation of the cells and cell spaces. In contrast to lamellar bone, the characteristic of this type of bone is a

lack of pattern. Now there is no standard nomenclature for this type of bone in the literature. Some authors do not mention it at all, others refer to it briefly as immature bone, primitive bone, coarse fibrillar bone, fiber bone, woven bone, nonlamellar bone and so on. We are indebted to Baker, a pathologist in England, who not only gave a good description of this type of bone but also believed that much of the confusion in bone pathology is due to lack of recognition of the differences between this bone and cancellous bone.

Let us use Baker's terms nonlamellar bone or woven bone for this type of bone. Where do we find it? This type forms the bony skeleton of the embryo. It is however gradually replaced by the lamellar type so that after the first year of life practically the whole of the skeleton is normally composed of lamellar bone. Sicher and Weinman believe that "this replacement of coarse fibrillar bone during the development of the embryo and in early postnatal life seems to be a recapitulation of a phylogenetic process. In the evolution of the vertebrate, coarse fibrillar bone is the first to develop and only the mammals elaborate lamellated bone".

This type of bone is also seen in many pathological states. In fact as you will see later I agree with Baker who states that any bone tissue formed rapidly under pathological conditions will be of this type. This type of bone may arise on trabeculae of bone or form in a mass of proliferating osteogenic cells. So sometimes one finds trabeculae formed partly of lamellar and nonlamellar bone, or in addition (slide 19) we see lamellar bone forming on nonlamellar bone. Sometimes one sees on a slide an island of woven bone. In fact, various combinations can be seen in pathological states. However, we have seen that eventually this nonlamellar bone is always replaced by lamellar bone.

Now let us examine the regenerations in the slow type of healing I labelled spontaneous regeneration. Slide 20. This case is after 18 months healing period. As I said before, the end result is the same as that following surgical intervention. Let us examine the growing tip. Slide 21. Here it is in higher magnification. This type of bone differs from other types in that the collagenous fiber system of the bone matrix is that of the original connective tissue. Besides the fibers, the cells of the original connective tissue are also enclosed. This type of bone is sometimes found in the bony insertions of the tendons and fasciae. In other words, then, this type of bone is formed around the connective tissue attachment in joints. This

then is the kind of bone one would expect to find in this location. Baker believes that this type of bone is formed by the metaplasia of existing connective tissue. He therefore terms it metaplastic bone. Again there is no standard nomenclature for this type of bone in the literature. In fact it is seldom mentioned.

Now let us look at a section (slide 22) a fraction of a mm into the bone and we see that this metaplastic bone also is replaced by mature lamellated bone, as is the case with woven bone. We can now make the following classifications:

Lamellar bone - compact, cancellous

Nonlamellar bone - woven, metaplastic

Summing up then, at various stages in the healing of a defect various types of bone are seen. Also cartilage may make a brief appearance. What does it all mean?

As I puzzled over these observations the thought came to me that the three types of bone suggest three different mechanisms concerned in maintenance and repair. If we could find out how the cells lay down the various types of bone we might learn something about the mechanisms. Let us start with lamellar bone.

I have always been fascinated by its remarkable structure. How is it formed? Slide 23. First, we have seen that lamellar bone has a very complex fiber system, quite different from any other skeletal tissue. Also, after the first year of postnatal life almost all bone in the body is lamellated. We know its formation is the last stage in the healing of a bone lesion. Another remarkable property was found by von Meyer, Culman and Wolff. Let me refresh your memories. Julius Wolff was a German orthopedic surgeon in Berlin in 1892. Sir Arthur Keith's account of his work is interesting. When Wolff was 56 years of age he published a great atlas monograph to which he gave the titled "The Law of Bone Transformation". He succeeded in rivetting the attention of thinking medical men on a mysterious property of living bone, namely, that its external form and its internal structure change with every alteration of function. In brief, a bone has the power to adapt itself to the burden it has to bear.

His work was partly based on some work in 1867 by two professors in Zurich: von Meyer the anatomist and Culman, and engineer and mathematician. Culman upon examining von Meyer's preparations and drawings of the neck of the femur, was convinced that in a mathematic and engineering sense,

the architecture of the femoral neck was a perfect mathematical design for the transference of weight. The trabeculae had been given the exact position and form which the expert mathematician and engineer would postulate for them. Later examinations of the structure of the femoral neck confirms Culman's conclusions. Sir Arthur Keith considered this a remarkable discovery. The osteoblasts are the architects of the femoral neck. They are the engineers who do their work according to exact mathematical law.

Having absorbed the Zurich discovery, Wolff began to extend his knowledge. First he studied sections of deformed bones and found that the internal structure was radically changed. He further found that these internal changes were followed by external changes in the external structure according to mathematical law. In other words the deformed bone became perfectly adapted to the change in function caused by the deformity.

His next finding was that if a normal bone was used in a new way its structure and form will change to meet its new function and thirdly, if a deformed bone is rectified, thus restoring normal function, the bone will reassume and maintain its normal shape and structure.

From the observations of von Meyer, Culman and Wolff then it appears that in addition to the fact that the formation of mature lamellar bone is the last stage in the healing of a fracture, the same process must be going on continually in the maintenance of bone. So not only is the microscopic structure of lamellar bone remarkably adapted (slide 24) to resist stress but macroscopically the material is laid down in struts and bars according to exact engineering design and principles. This apparently remarkable engineering power of osteoblasts, as bone builders, has aroused the interest of a number of investigators since Wolff's time. Goodsir, Denby, Nicholson and later Woodlands investigated this problem by studying the scleroblasts which form the skeleton of sponges. It seems that scleroblasts can also build calcified tissues with exact engineering design. So the suggestion was made that scleroblasts and osteoblasts have an inherited sensitivity to stresses, I suppose comparable to the inherited ability of nerve cells for conduction. Sir Arthur Keith calls the scleroblasts "the ancient ancestral relatives of the osteoblasts".

Now this behaviour of the osteoblasts in building lamellar bone, besides being fascinating, cannot be overlooked if one seeks to understand the mechanisms concerned in the maintenance and repair of bone. So let us see if

if we can explain the bone-building mechanisms in terms other than an inherited skill of the osteoblast. In addition to being laid down to exact engineering principles, we must also account for the ability of lamellar bone to continually adapt to varying amounts of stress. If the stress is increased more bone is laid down, and vice versa if the stress is decreased.

Well, let us see how bone is formed. The first sign of bone formation is a condensation (hyalinization) of the semifluid homogenous ground substance of the tissue between and around the fibrils. This change in the chemical composition of the ground substance leads to a masking of the embedded fibrils, because the ground substance and fibrils now have nearly the same refractory index. The organic substance thus formed is called osteoid tissue. Shortly after the osteoid tissue becomes calcified. It is important to remember that bone formation consists of two distinct phases:

1. the formation of the matrix in which the fibrils are embedded, and
2. the calcification of this matrix.

Now let us consider another important difference between bone and uncalcified tissues. In bone formation the osteoblasts secrete or form around themselves the typical organic intracellular substance which when calcified becomes bone. Osteoblasts have many long protoplasmic processes which Dr. Ham likens to the legs of a spider. Using his phrasing, when osteoblasts lay down the organic intracellular substance of bone they do so not only around their cell bodies but also around their processes. Thus canaliculi are formed through which the osteocytes are nourished. Dr. Ham also pointed out that this mechanism from the capillary to the cell by which food and oxygen are brought to the bone cells is inefficient. Thus the majority of osteoblasts are not more than 1/10 of a mm away from a capillary. Therefore as new layers of bone are deposited on bone the cells of the deeper layer become farther and farther from their capillaries and die. When the cells die the calcified bone matrix is resorbed. Why the persistence of a calcified intracellular substance should depend upon living cells is not understood. Then again, there is the problem of the life span of the osteocyte. Osteocytes cannot divide in bone as in cartilage so when they die of old age or from other causes, the cells can be replaced only by the complete breakdown of the bone and the build-

ing of new bone. Thus we see that to maintain a calcified bone tissue nourished by living cells there must be a continuous proliferation of osteoblasts to form new bone. There must also be continuous resorption of bone. We see also that the growth of bone automatically causes the death and resorption of bone.

Now one more fact. Slide 25. In this section at 28 days we see a new growth of nonlamellar bone. Here growing under the same conditions we see lamellar bone being formed on it. Or are the conditions exactly the same? The only difference I could see was that the lamellar bone was laid down on an existing bone surface. Then I realized that lamellar bone is always laid down on an existing bone surface. Only woven bone can arise in a mass of proliferating osteogenic cells. Of course nonlamellar bone may also be laid down on existing bone. I therefore took the data to Dr. Wrenshall, showed him this slide and asked him how this pattern could be formed. I emphasized that the organic matrix of fibrils and ground substance was laid down first and then calcified. I explained that the orientation of the cells and fibrils along the lines of force was understandable as that always occurred when fibrous tissue was exposed to stress, example tendon. The puzzling feature was that in alternate sheets the general direction of the fibrils changed as a rule through an angle of 45° , though sometimes through a complete right angle. Stress appeared to be a factor. Could stress form the pattern? Here is what came out of the discussion.

Cheese cloth is a material composed of fibrils. Slide 26. See what happens to the fibers when a shearing force is applied. If a tendon was formed and then attached to bone on one side with the other side free, the conditions are present where the tissue may be exposed to a shearing force. Lamellar bone is only formed when new bone is added to an existing bone surface so it may be affected by a shearing force before it is calcified. In this connection Sicher and Weinman report "there is only one location in which bone is exclusively under tensile forces", namely in the innermost layer of the dental alveoli to which the suspensory fibers of the periodontal membrane are attached. Here the direction of the fibrils is not subject to a regular change from layer to layer and thus, lamellae of the bone are indistinguishable".

Now we have seen a possible explanation of the manner in which part of the microscopic structure of

lamellar bone is formed. But how is lamellar bone laid down according to exact mathematical law? Again, how is it continually adapted to varying degrees of function? Also we often see bone added as woven bone with an entirely different microscopic arrangement. When bone is added to bone why is the pattern sometimes lamellar and sometimes woven? In addition to these questions there are other observations that must be explained but we will disregard these for the moment. How are these well-established observations brought about? For the present let us consider the mechanisms by which lamellar bone is laid down. Later we can consider woven bone.

A study by Weiss throws some light on this mechanism. He demonstrated that if a thin film of fibroblasts grown in tissue culture is subjected to regional tension, the cells exposed to the tensile force (1) multiply more rapidly and (2) orient themselves in parallel lines in the direction of the strain. In other words, tension appears to be a mechanical stimulus determining directly or indirectly not only an increased product of fibrous tissue but also the direction in which it is deposited. LeGros Clark believes that "there is a reason to suppose that the tensional forces probably do not act directly on the fibroblasts but they influence the degree and direction of their proliferation by modifying the ultra structure of the substratum in which the cells lie."

Now let us postulate the result of an increase in stress on bone. The exact mechanism of such stimulation remains obscure, but it seems probable that the bending movement in the bone structure affects the substratum of the cells at the bone surface in such a way that the proliferation of these cells is stimulated in a similar way as the effect of stress on fibroblasts. Thus the osteoblasts would proliferate faster at the point of greatest bending. With normal resorption then the bone structure would gradually assume the form dictated by the direction of the stress. There are many observations that would support this view. For example, in longstanding rickets or in osteomalacia where the calcification of the osteoid tissue is delayed, the trabeculae of the cancellous bone is thicker than normal owing to the increase of lamellar osteoid tissue on their surfaces. Turnbull believes that there is little doubt that this is the result of normal stress on ab-

normally soft bone. Note that the osteoid tissue has the lamellar structure. However, some authors believe that this phenomenon is caused not through increased deposition but through reduced resorption.

But we still have to explain why bone is added to a trabeculae as lamellar bone or may be added as nonlamellar bone. Also trabeculae may be observed composed of lamellar bone on which is built nonlamellar bone. Let us suggest a mechanism consistent with the facts with lamellar bone first and deal with nonlamellar bone later. When bone is exposed to increased stress or stress from a different direction, the bending movement of bone stimulates the proliferation of only the cells in contact with the bone. I have sketched a trabeculae of bone that is to be strengthened at point A. The cells in this area proliferate from the stress stimulus forming a seam of osteoid tissue. The cells at point B, further away from bone, are not stimulated by the bending of the bone. The stress stimuli for the formation of an additional seam of osteoid tissue would not be transmitted to form a second seam of osteoid tissue until the first seam was at least partly calcified. This explanation is consistent with the observed facts about the growth of lamellar bone. Also we will see later, when osteoblasts differentiate some distance away from a bony surface, the bone formed is not lamellar but woven bone. In fact, when bone is laid down anywhere except by small additions to a bony surface it is always woven bone, for example, in the embryo. It is well known that lamellar bone is only formed slowly on an existing bone surface. However we will see later that from the clinician's point of view, the important thing is not whether the mechanism postulated is altogether correct. He is mostly concerned with whether or not functional stresses are (1) the stimuli for the continuous proliferation of cells required to maintain lamellar bone, and (2) are the stimuli responsible for the continual adaptation of bone to changes in both the amount and direction of the functional stresses. Thus it is important to bring out the evidence that such a mechanism can exist.

Now let us see how woven bone is formed. We have mentioned its occurrence in the bony skeleton of the embryo and in many pathological states. Let us consider a fracture an example of a pathological state. One of the striking features in fracture healing is the almost wild proliferation of the osteogenic cells. They proliferate so fast that differentiation lags behind. Not until diff-

ifferentiation occurs do the cells start to form osteoid tissue. By the time differentiation takes place they may be some distance from the bony surface. Slide 27. We can see this phenomenon in the next slide. Slide 28. Notice that the cells have differentiated a considerable distance from the bone. The osteoid tissue thus formed is therefore not affected by the stresses as was the situation in the formation of lamellar bone. Also, of course, a fractured bone can not be subjected to stress. So we see the reason for the lack of pattern in the fibrils and bone cells in the newly formed woven bone.

We see then that in repair the stimuli for the proliferation of osteoblasts to form new bone is different from that in bone maintenance. So let us term the formation of lamellar bone the maintenance mechanism, and the formation of woven bone as the reparative mechanism. For convenience we can then term the stress stimuli physiological stimuli and the stimuli in repair as pathological. Thus it seems logical to talk of maintenance as a physiological process, and repair as pathological.

We see the reason why woven bone is the type first formed in a fracture. Here, not until continuity is established bridging the gap is the newly formed bone subjected to stress. By the time that happens the healing process has been completed and the pathological stimuli which cause the cellular proliferation for the woven bone, have subsided. The establishment of continuity permits the maintenance procedures to take over and the woven bone is gradually replaced by lamellar bone, the required proliferation of the cells for the reconstruction of the bone being from stress stimuli. If, however, union is not attained before the pathological stimuli subside, the union is fibrous. But let us leave a discussion of that until later.

Our concept of the manner in which lamellar and woven bone are formed is supported by many observations. For example we see the reason why the bone of the embryo is woven bone and why in the first year of life it is replaced by lamellar bone; why bone cannot be maintained unless it is subjected to stress but can be repaired without stress, in fact stress must be eliminated. These facts and many others suggest two mechanisms. Again, Sir Arthur Keith appeared to be greatly impressed by the ability of the osteoblast to lay down bone according to

exact mathematical law. He wrote quite a lot about it and made an interesting observation. He wrote: "In Paget's disease we see quite another disturbance in the life of the osteoblasts. They are no longer acutely sensitive to the stresses which fall on the skeleton. They lose their engineering qualities and lay down their materials clumsily." According to our concept this phenomenon occurs when bone reacts to the pathological stimuli rather than the physiological or stress stimuli. So I looked up the histopathology of Paget's disease and sure enough, the new bone formed in an active area is of the immature or woven type. Dr. Sapper's slide on the pathology of Paget's disease was interesting to me as the bone shown was all woven bone. Now, I am not a pathologist and do not wish to discuss bone diseases further in this paper but it seems to me that further understanding of the physiological mechanism of bone should be useful in studying interference in those mechanisms which result in pathology.

We have seen how lamellated and woven bone are formed. Slide 29. How is this metaplastic bone commonly found in the bony insertions of tendons and fasciae formed? Naturally to study this problem I turned to the part of the skeletal system I know best, the maxilla and mandible. The next part of my talk deals mostly with dental structures but I am only using these tissues to study basic principles. Later we can apply the principles to other parts of the skeletal system.

In studying the growth of the skull it is commonly divided into two parts, the cranium and the face. There are a number of reasons for this, i.e. the cranium increases its size by four times from birth to the adult, the face about twelve times. Again, the growth of the cranium is nearly completed by the 8th year while the facial part of the skull continues to grow even after epiphyses in the long bones are closed. This situation makes the growth of the skull quite complicated. So to simplify matters let us refer only to the part that the mandible takes in facial growth. Hunter's work on the growth of the mandible by feeding of madder to animals is a classic in bone physiology but I do not wish to refer to that type of growth, (Slide 30) but rather to the growth in the length of the ramus. In this sketch two stages in the growth of the mandible are superimposed. The increase in the length of the ramus occurs largely by interstitial growth in the cartilage in the condyle; not exactly as growth at the epiphysis occurs in long bones, but for our purposes the

difference is not important. Slide 31. I just wish to point out that the growth of the ramus in length tends to move the lower teeth away from the uppers. Now how are the teeth kept in contact? Unless the teeth extrude from the jaws all the joints must grow upward to compensate for the increase in length in the ramus. How then is the growth in this alveolar part of the jaw exactly co-ordinated with the growth at the condyle? Slide 32. Also, in this slide, we see that in addition in normal development each tooth keeps the same relation with its opponent. So the adapting mechanism must work in the horizontal and lateral direction as the face grows in size in all directions. But for simplicity let us consider only the mechanism by which the growth in the alveolar bone is exactly co-ordinated with the growth at the condyle as it operates in the growth of the face in the vertical direction. This sketch shows a normal dental joint attaching the molar to the mandible. We know that this tooth is subjected to force from its opponent in this direction. Therefore as one expects, most of the connective tissue fibers are arranged in this direction to sustain this force. And vice versa if we examine a section of the joint noting the direction of the fibers we could postulate that the force came from this direction. Slide 33. Now let us look here. We see fibers running in this direction. This indicates there must be a force tending to pull the teeth in the opposite direction, otherwise these fibers would not have developed. Again we see a group of fibers arranged in a horizontal direction, so there must be forces in that direction also. If we examine the joint diagonally opposite we see the same grouping of fibers opposing these. From what has been said about lamellar bone, we see that in effect the tooth is held in position by the forces to which it is subjected. I have attempted to illustrate this situation by this little gadget. (Illustrate) In tissue, however, the adaptation takes time. We know that if we change these forces by adding a force here with a spring, the tooth and the joint will grow in this direction. That is how orthodontists move the whole joint into a new position by establishing new forces. The moving process is carried out by the maintenance mechanism if done correctly. Thus, as the ramus grows in length the balance of the forces will be reduced in the vertical direction as the tooth is moved from its opponent. The resulting extra pull on these fibers stimulates a more rapid bone growth where the fibers pull

harder on the bone. Here we see the new bone. The same situation will occur at the apex of the tooth. The whole joint will grow upward until the balance of forces is again reached by contact with its opponent. Now, as the osteoblasts proliferate in this direction into the connective tissue, naturally the connective tissue fiber system will be enclosed in the bone, which is the situation we saw in the spontaneous type of healing. Slide 34. Once calcification occurs this new bone then becomes subjected to the deposition and resorption mechanism required to maintain a calcified tissue nourished by living cells. It is also subjected to stress so the result is lamellar bone formation, the same end result as occurred when woven bone was formed after surgery. So you see we have an automatic mechanism designed to keep the tooth in function and in the correct relation with its opponent during all the changes that can occur in the growth of the face. But more than that, this same mechanism also provides for the continuous automatic adaptation of the structure to function so essential for the health in skeletal tissues. Increase the forces and we increase the amount of structure, both connective tissue and bone and vice versa as the forces are decreased. We see then when the balance in forces is changed the whole joint tends to move into a position which is the resultant of the changed forces. We will see later that an imbalance in forces may also be caused by a change in structure. Such an imbalance will also tend to cause an adaptive reconstruction of the joint.

The movement of the tooth by the use of springs or other devices which produce a pressure has generally been considered by orthodontists as being accomplished by controlled pathology: a pressure resorption of bone, etc. According to our concept orthodontists are making use of a physiological mechanism. Such tooth movement, or should I say joint movement, is then a physiological process. I believe the implications of such a concept are basic to orthodontists but perhaps a discussion of these implications would be out of place here and should be left to a dental audience.

One essential factor in this dental joint mechanism is a balance of forces. Now I disturbed this balance in my experiments by removing part of the structure, but I did it in a special manner. Let me explain.

About this time I began to realize that I was possibly dealing with a type of automatic system. I did not know

much about this subject so I decided to find out something about the nature and characteristics of automatic systems. I obtained a book on the relatively new science underlying the development of automation, a word we hear about these days. I found that while automatic control is not a new thing, the development of the science, the formulation of the underlying principles and the far-reaching application is a very recent achievement. Another thing that is new is the growing comprehension of the universality and power of the self-regulating principle.

It is now recognized that the self-regulating mechanisms are an inherent feature of innumerable processes in nature, living and nonliving, although in the past the characteristics of self-regulatory mechanisms were often mistaken for the operation of some conscious design or force.

I found that there are different types of self-regulatory mechanisms with different characteristics; that they have different behavior patterns and properties. For example the closed loop system is capable of various self-excitatory types of behavior. Life itself is a self-regulating mechanism. Another factor of particular interest to the physiologist is "that studies of the behavior of automatic control systems permit a new insight into a wide variety of happenings in nature and human affairs". As Arnold Tustin states: "The notions that engineers have evolved from studies of automatic control systems are useful aids in understanding how a man stands upright without toppling over; how the human heart beats; why our economic system suffers from slumps and booms, why the rabbit population in parts of Canada regularly fluctuates between scarcity and abundance."

Now if, as the evidence suggests, the mechanisms involved in the maintenance of skeletal tissues are self-regulatory in nature it should be advantageous, perhaps even necessary, to study and think of them as types of automatic control systems. As I considered this hypothesis it appeared more and more logical and I proceeded on that basis. I will be using terms such as interdependence, developed in the science of self-regulating mechanisms. In order that we may be thinking along the same lines I shall quote briefly from an essay by Arnold Tustin. The chief purpose of his essay is to make clear the common pattern that underlies the phenomena I have just mentioned and many other varied phenomena. He states "The common pattern is the existence of feedback, or to express it more

generally, "interdependence". He explains interdependence by referring to the automatic household heating plant where the hookup of output to intake brings about a radical change in the character of the system. The familiar chain of cause and effect - fuel, fire, heat - is closed into a loop of interdependent events and the heating system has become self-regulating. Tustin goes on to say that "all controlled and regulating systems depend on common principles." We should not be able to live at all, still less design complex control systems, if we did not recognize the relationship between events - what we call cause and effect. When the room is warmer the thermometer on the wall reads higher - we do not expect to make the room warmer by pushing up the mercury in the thermometer. Now consider the case when the instrument upon the wall is not a thermometer but a thermostat, contrived so that as its reading goes above a certain setting, the fuel supply to the furnace is progressively reduced and conversely, as its reading falls below that setting, the fuel flow is increased. This is an example of a familiar control system. Not only does the reading of the thermostat depend upon the warmth of the room, but the warmth of the room also depends on the reading of the thermostat. The two quantities are interdependent. Each is a cause and each an effect of the other. In such cases we have a closed chain or sequence, what engineers call a closed loop. Such a system has certain behavior patterns and also certain potentialities.

In the automatic mechanism we have been describing in the maintenance and continuous adaptation of the dental joint we see that cause, the functional forces and the effect, the building of the tissues, are interdependent, each is a cause and each an effect of the other. That is why an imbalance can result from change in either factor, the forces or the structure. But, automatic regulation can operate only as long as the system is working. For example, in a household heating plant if the thermostat becomes defective or the power goes off or we run out of fuel, the automatic system no longer functions; we no longer have a self-regulatory mechanism. The system must be repaired first. Now, if I had damaged the structure of the dental joint in such a way that the self-regulatory maintenance system could not operate, I could not expect regeneration. But I did it in such a way that the system could still operate. Regeneration of the joint could then occur by the same mechanism by which structure is adapted to a change in function. The lost structure would then be replaced. This can only occur if the automatic system is working. In other words, if I disturbed

the balance in forces by removing a part of the structure, as long as the self-regulatory system was operating the balance could be restored by a rebuilding of the structure. Basically it is the same mechanism by which a balance is restored when the orthodontist creates an imbalance by means of his springs, or an imbalance is caused by an increase or decrease of function. Of course we must remember the limiting factors in time and degree. Obviously too sudden or too great variation will not be tolerated. Let us see how one type of lesion interferes with the automatic self-regulatory system while another type does not interfere.

After I first obtained the spontaneous type of joint regeneration, I knew only that such regeneration could occur. Even though I did not know how it was brought about, I tried it in my practice and was delighted with the results. But as time passed I found that even in the same mouth the regeneration occurred around some teeth while others showed no improvement. It was a hit or miss affair. Gradually I realized the type of damage which did not respond to treatment. Slide 35. This slide illustrates this type of damage. The dental people will recognize this as an intrabony pocket. The term is used to describe a lesion where part of the wall of the pocket caused by destruction of the joint is of bone rather than of completely soft tissue. Slide 36. The next slide is a section of intrabony pocket. We see that the epithelium has proliferated and covered the detached tissue. This bone is maintained by the attachment to the next tooth. With this type of damage the fibers can not be attached to the tooth and the bone, therefore there can be no spontaneous type of regeneration. In other words the automatic system can no longer operate in this area. From what has gone before we can see that before normal maintenance mechanism can operate, continuity between bone and tooth must be established in this area as the stress stimuli are transmitted from the teeth to the surrounding tissues. The re-establishment of continuity must be carried out by the reparative process which we will discuss later.

We see then that we may have two types of lesions of the skeletal system: (1) where the self-regulatory maintenance system can still operate to repair the damage, and (2) where the system is interrupted and cannot operate until it is first repaired. It is obvious that the treatment of each type will be basically different.

Before we leave the maintenance system to discuss the reparative system I would like to briefly consider another way in which the health of the skeletal system can be interfered with by disturbances of the self-regulatory mechanisms. The two interdependent factors are:

- 1) the functional force, and
- 2) the building of tissues in response to these forces.

As each is a cause and each an effect of the other, the system can be disturbed by changes in either. Let us consider the response of the tissues. The most common disturbances are: (1) endocrine malfunction as in diabetes; (2) nutritional as in scurvy or rickets, and (3) bone disease, such as the various types of osteitis, etc. Interference in tissue building may also interfere with or change the function as occurs in various bone deformities, i.e. bow legs. One could go on discussing the implications of interdependence in etiology diagnosis and treatment of disturbances in the skeletal tissues with profit, but we must get on.

Now, what happens if the automatic control system is not restored? As one would expect, if the system by which function and structure are co-related is not functioning, there would soon be an imbalance between function and structure. The result would be a chronic type of trauma.

I would imagine that the orthopedic people would encounter such chronic trauma quite often clinically. If so, a discussion of this problem might be of interest. But I will only refer to it briefly in my own field. The dental joints are particularly susceptible to chronic trauma. I use this term to differentiate from the acute type of trauma which results in a fractured jaw. The reason is that the dental joints are susceptible to interference in the operation of the automatic maintenance system by disturbances in either of the interdependent factors, generally both. In this part of the skeleton of the masticatory apparatus, functional disturbances and disturbances in the response of the tissues of varying degrees are the rule rather than the exception in man's environment to-day. Sooner or later the nervous system becomes involved and there is pain. Often, when the trauma is not too severe, the pain is of the referred

type, usually in the same segment. I mention this as certain ideopathic head pains seem to originate in the masticatory apparatus. At least they disappear dramatically when the dental condition is rectified. My remarks on pain are based on clinical rather than experimental evidence so let us consider them a footnote. These remarks were made to satisfy the curiosity of some of the members of our staff who wanted to know the basis on which quick relief resulted from a simple treatment in cases involving themselves or their families. This whole subject of chronic trauma is fascinating but we must leave it and get on.

Summing up then, we have seen that to treat a lesion of the skeletal system our ultimate objective is to restore normal operation of the self-regulatory maintenance system. When this is operating, function and structure are interdependent, each is a cause and effect of the other, therefore beautifully balanced and adapted to each other. We have seen that this balanced state of affairs may be upset by changes in either of the interdependent factors, the function or structure. The system is capable of restoring itself with certain types of disturbances but not with others.

Now let us consider the application of this concept to lesions of the skeletal system. For our example let us take two types of traumatic lesions, one involving bone - a fracture - and one involving connective tissue - a torn ligament. Traumatic lesions of this type are probably the least complicated breakdown of the self-regulatory mechanism. Consider the bone fracture first. What has happened from the viewpoint of self-regulating mechanisms? As is the case when the thermostat is broken in a self-regulating heating system, following the fracture the automatic maintenance system is no longer operating. The stress stimuli cannot function again until continuity is reestablished. But new bone must be formed, to establish continuity. As stress stimuli are not available the required proliferation of cells must be induced by other types of stimuli, those operating in the reparative process. In the lower forms of animal life whole organs or limbs may be regenerated after wounding. Man has not this capacity but does possess a remarkable capacity to restore continuity to an injured area. This is usually accomplished by reattaching the parts by connective tissue. However, in a fracture this is an undesirable method of restoring continuity. The surgeon takes care to

promote a bony union. But as you know, with certain types of injuries, difficulties in inducing bony unions often occur. References to our work on the fibula of dogs might help in considering this problem. We removed a section of bone with its periosteum from the fibula leaving a gap about 2 cm in length. If such a lesion is allowed to heal in the ordinary way the result will always be a fibrous union. In fact, continuity will be reestablished by the formation of a tendon. Now the clinician desires bony union. What are the determining factors in inducing a bony rather than a fibrous union? We have been able to induce bony union in a number of cases as shown in the next slides. Slide 37. Here all that was done was to bridge the gap by means of a polyethylene tube. How did the tube produce a bony union instead of a fibrous one? Another series of five cases were done exactly the same way except holes were drilled in the tubing. In all cases the union was fibrous. You might say 5 cases were not enough and I agree, more should be done but I am reasonably sure the result would be the same in 100 cases, with the perforated tube as there is a characteristic type of healing. Still, these conclusions should be checked by further cases. How can these findings be interpreted? It is fairly well established that osteogenic cells have the potency to differentiate to either osteoblasts, fibroblasts or chondroblasts. But the evidence that fibroblasts proliferating in from neighboring connective tissue have this genetic potency is not nearly so good. One might interpret the evidence with the perforated and intact tubes by saying that the perforation permitted the gap to be filled by fibroblasts from the side of the wound, thus the resulting fibrous union. The intact tube allowed only osteogenic cells to fill the gap from the ends of the bone, resulting in bone. This is one interpretation. But another interpretation would be equally consistent. Possibly for osteogenesis there must be a certain environment such as a certain pH or lime salts concentration. The intact tube permitted the establishment and maintenance of this environment while the perforated tube did not. In other words, the tube may function by keeping something out or something in. Again, in our successful cases shown previously where woven bone was induced to grow around the tooth, we planned our surgery to prevent fibroblasts from proliferating into the wound and to permit the osteogenic cells to fill the gap. In some cases, the detached periosteum was used for this purpose, in others the epithelium was used as we see in the next slide. Slide 38. But again, the periosteum or the epithelium might have functioned like the polyethyl tube by keeping something out or

something in. Most authorities agree that the periosteum is important in bone healing. In view of these experiments how does detached periosteum function? It seems to me that to rationally plan to induce bony union in those cases where it is known from experience that a bony union is difficult, further light should be thrown on these basic problems.

(1) the type of cell that proliferates into the lesion required for osteogenesis

(2) the environment required for osteogenesis

As I studied bone and connective tissue repair the evidence suggests that in the reparative process as well as in maintenance, we are again dealing with an automatic control or self-regulating mechanism. You may ask what is the significance of this statement. Again may I suggest that if a mechanism is of the self-regulating type it is advantageous, perhaps even necessary to deal with it on that basis, from the viewpoint of either the investigator or the clinician. This seems to be the case from what I can learn about dealing with self-regulatory mechanisms in other fields in nature or in human affairs. Many blunders have been made by the linear type of thinking from cause to effect rather than the recognition that in self-regulating mechanisms the various factors are interdependent, both cause and effect. I suppose an expert in the science of automatically controlled systems could tell us the importance of recognizing if an unknown mechanism is or is not self-regulatory. If it is, the importance of investigating the scheme of dependence, how the various factors are determined by one another and by disturbances outside the system, etc. But most of us are not experts in this new science so let us see for ourselves. To do this let us review briefly the sequence of events in restoring bony continuity following the removal of a 20 mm section of bone with its periosteum from the fibula of a dog. In this case a polyethyl tube filled with a graft (small chip) was used. Most of the slides are at the 28th day of healing. Slide 39.

As we consider the sequence of events let us look for some of the self-regulatory features, two types in particular; (1) the initiation of each phase appears to be automatically provided for in the preceding phase; (2) the occurrence of each phase automatically provides

for its own cessation. For example, let us consider if initiation of the proliferative phase is provided for in the previous exudative stage and is automatically stopped by its own completion.

First there is the inflammatory or exudative stage. One feature in this is the formation of a blood clot. Here is the blood clot still in the centre of the tube. Slide 40. Let us say it represents the exudative stage.

Slide 41. If the mechanism is self-regulating, conditions for the initiation of the next phase, the proliferative, should be provided for in the exudative stage. Slide 42. Here we see the cellular proliferation still going on after 28 days and we know normally it will continue until all results of the exudative stage are disposed of. The phase of cellular proliferation initiated by the exudative stage will automatically stop when the gap is filled with new tissue, as with the filling of the gap the pathological stimuli cease.

Then comes the differentiation of this new tissue to form bone. The first step in this phase appears to be resorption. The conditions for initiating the resorptive stage should have been already prepared in the preceding phases. We know that is the case with some conditions that produce resorption. We believe resorption is a cellular process, therefore blood supply must be present. That is provided for by the proliferative phase. Secondly, the presence of calcified tissue that is not nourished by living cells seems to initiate resorption. We know that the osteocytes die for some distance from the line of fracture. May I suggest that grafts may aid osteogenesis in larger defects by providing additionally for the resorptive stage. We also know that if the proliferative phase fails so does the resorptive stage, with the dead bone of the grafts becoming sequestria.

Next comes the differentiation of osteoblasts and the deposition of bone on the previously resorbed surfaces. There is evidence which we will see later which suggests that the differentiation of osteoblasts will peter out unless preceded by the resorptive stage. After the osteoblasts differentiate and lay down bone over the grafts eventually the grafts get completely covered by new bone. The covering of the grafts automatically stops their further resorption. About the time this occurs, the same cells that had previously differentiated to osteoblasts now differentiate to chondroblasts. This may not be merely

coincidence. It could be a self-regulatory feature, resorption providing a factor for the differentiation of osteoblasts and the formation of new bone. The formation of new bone automatically stops resorption, giving a type of self-regulation of deposition and resorption, a necessary control in bone metabolism.

Slide 43. Here is the new cartilage. Slide 44. Later stage. However, it appears that the conditions here are not suitable for the maintenance of cartilage, as the chondrocytes die and the cartilage becomes calcified, another self-regulatory feature. If cartilage is not used as cartilage it automatically dies.

Slide 45. Again the presence of dead calcified tissue initiates the resorptive stage and the cartilage is resorbed.

Resorption is again followed by a wave of bone deposition. If the cartilage does not calcify, it is not resorbed and I believe there would then be no further wave of deposition.

As more and more bone is laid down the resorption and deposition necessary in bone metabolism will continue.

When union or continuity is re-established, the reparative process is over. The next step is the adaptation of the new bone. With continuity established, the stimuli by the stress mechanism automatically take over and the adaptation of the new bone to function proceeds. May I suggest that non-union of fractures may not be the absence of healing per se but may be the failure to set up the self-regulatory system for new bone formation in the area between the bone ends.

Our purpose in outlining the steps that occur in the healing of a bone defect is to illustrate a self-regulating system and secondly, to get some idea of the significance of bone repair as a self-regulating system. When studying automation I noticed that it was brought out that self-regulating principles must be used in certain chemical industries as the chemical processes are too complex to be controlled otherwise. It seems logical that the self-regulating principles have a large part in the living processes. How does the concept developed help us? One could think of many ways in both application

and in pointing out new research. Let us consider two.

The way to further research on certain clinical problems is indicated. As one studies the reparative system, the steps where failure is most likely to occur become apparent. Interference may occur at any step but it would appear that bone is more susceptible to failure of the proliferative phase than is the case with uncalcified tissues. In the proliferative phase the defect should be completely filled with cells. If this does not occur we of course do not achieve union, any grafts used become foreign bodies, the defect is filled with fluid and susceptible to infection and further necrosis of bone. In a self-regulating system the fault probably occurs in the preceding exudative phase. In the concept of an automatically controlled mechanism the interference must be with something that regularly occurs in the exudative stage following fracture. For example, one thing that regularly occurs would be the formation of a blood clot. Perhaps one function of a blood clot is to provide the stimuli for cellular proliferation. We are all aware there is evidence that a blood clot has a place in wound healing. If the initial blood clot is disturbed it is quite possible, especially with bone, that a new clot would not form. This brings up the question, what elements in a blood clot initiate the proliferation? It could be in the cellular components, the plasma or the serum. There are methods already developed that I believe would be suitable for investigation of this basic problem.

Another cause of failure in the proliferative stage may result from interference with the response of tissues to the stimuli. For example, our tubes were made to fit the fibula loosely to permit cellular proliferation which raises the periosteum. By mistake a number of cases were done with a tight fit over the periosteum. No tissue grew into the tubes, just fluid, and most of the cases became badly infected. It may be significant that in the other leg of the same animals where proliferation was not interfered with, not one leg became infected. We had a similar result in cases where the tube was not filled with blood. These cases are observations in our experimental work rather than controlled experiments. I would like to do controlled experiments. Consideration of Truetta's work on fractures from this viewpoint is interesting. Perhaps the good results were not due to the war of the bacteria.

Another step susceptible to failure in bone healing is differentiation. Slide 46. Here is a defect where the proliferative stage was successful but differentiation did not occur. A little new bone was formed but it soon petered out. However, differentiation did occur along the cells contacting a calcified surface, the tooth, which had been resorbed earlier in the healing process. Slide 47. Here is the resorptive stage. Slide 48. Notice the amount of resorption in a healed case that persists, as dentine is a nonvascular tissue. Slide 49. The same type of wound at 30 days where resorption was provided for by the use of grafts. Slide 50. Here we see resorption prior to the differentiation. Here the resorptive stage is over and deposition of bone is occurring on the previously resorbed surfaces. I am showing these slides as some of the experimental evidence to support the concept of interdependence between the events occurring in bone repair.

This brings up the question of the role of grafts as aids in osteogenesis. Let us consider them in the light of our concept. Three types of grafts are in common use: compact, autogenous cancellous, and bone from bone banks. Take them in order. We bridge a gap with a nicely fitted compact graft. Continuity could be more quickly established by the bone uniting with the graft at both ends. This union is similar to that occurring between the bone and the calcified cartilage on the diaphyseal side of the epiphyseal plate. With the establishment of continuity the maintenance mechanism can now operate. The situation then differs only from the normal in that there is more dead bone to be replaced. We know that normal osteocytes die, and the dead bone is resorbed and replaced by new bone. The disadvantage of course is that the replacement process is slow. Until replacement with living bone has taken place the strength of the repair depends on dead bone which tends to become more brittle. However, steps might be taken to speed up the process, also to decrease the danger of a fracture before the graft is replaced by living bone. You might notice that the compact graft probably fulfills the function of the tube, also the requirements for initiating the reparative mechanism to assure union of the graft and bone by the formation of woven bone. A poorly fitted compact graft might not do this.

Again, a compact graft might be useful in the treatment of some fibrous unions. This sketch illustrates

a fibrous union. Continuity could be established by an onlay of compact bone. With the establishment of the maintenance system, remodelling according to function could occur eliminating the fibrous union. I believe that this has been demonstrated experimentally.

Now, autogenous cancellous grafts. Slide 51. This slide is of a case where autogenous cancellous grafts (small chips) were packed into a gap in the fibula of a dog. No tube was used. Slide 52. Here it is in high power. Notice the considerable amount of woven bone formed of one chip at 8 days. It appears that some of the covering and lining cells survived and are stimulated to proliferate by the same pathological stimuli that initiates the proliferative stage. Providing specialized cells in the defect eliminates the necessity for the first resorptive stage that occurred in the tube. In the tube the covering and lining cells of the graft died as they were prevented from connecting up with the nutrition from the host by the tube. Thus we should expect that healing where specialized cells survive would be faster. In addition the grafts act as islands of osteogenesis all along the defect. It would be expected then that replacement would be faster if the defect was of the type where specialized cells survived the graft. Slide 53. Here we see this is the case. On one side is a tube with grafts and on the other side autogenous grafts only. In certain types of defect autogenous grafts could have certain advantages. However, with the exception of the first resorptive phase the sequence of events from then on is the same as occurred in the tube. Autogenous grafts in suitable defects may thus serve two functions in promoting osteogenesis. For those people wishing further information about the survival of specialized cells I would suggest reference to the work of Drs. Gordon and Ham.

The covering and lining cells in cancellous bone from a bone bank would not be expected to survive. I have not used this type of graft experimentally but the evidence suggests that it may function in the same way as grafts in the tube which also die. However, I would be surprised if cancellous bone from a bank would have any effect in promoting union across the gap in a fibula without the use of a tube. I hope to run a series of cases to test this as it would appear to be an illuminating experiment for investigating the nature of the reparative mechanism. Theoretically, healing would be slower with bank bone than with autogenous grafts in those defects where a consider-

able number of specialized cells could survive; there would be less difference in those defects where specialized cells in autogenous grafts could not survive grafting. In any case regardless of what type of graft is used, if union occurs, the end result is the same.

There is another phase in bone healing I would like to touch on but it can be illustrated by considering our second example, the traumatic lesion, in connective tissue, the tearing of a ligament. Slide 54. This is the slide showing the skeletal system of a foot in the normal. Slide 55. Here the ligaments have been torn on one side. In talking to my medical friends their rationale of treatment is essentially as follows: in such a condition it is obvious that the ligament must rest to have a chance to heal. Therefore it must be supported or immobilized to some extent until healing takes place. They seem to believe that the reason healing is slow in a tendon is because it has a poor blood supply. There seems to be some disagreement about the degree and duration of immobilization. Is this rationale of treatment adequate?

Let us look at this situation from the viewpoint of a self-regulating system. Our treatment is designed to restore the part to a state of health, that is, to restore the operation of the self-regulatory maintenance system. It is interesting to note the similarity between the healing of a tendon and the healing of a fracture.

Before the accident, the automatic maintenance system was operating with all the implications for the joint the automatic system implies. After the accident the automatic maintenance system cannot operate, until continuity is again established in the injured area. To do this, new tissue must be formed. The stimuli for the cellular proliferation is of the pathological type automatically produced locally by the wound. Once the defect is filled with new tissue the pathological stimuli subside. The maintenance system then takes over. The tissues must be strengthened and adapted according to the function. The stimuli for the required cellular proliferation for this phase are stress stimuli. The process is completed when the functional structural harmony or balance is at last re-established.

I could have used the same words in describing the situation with a fractured bone. But let us go back a minute. In fracture healing, to permit all these steps to occur it was essential to immobilize the injured area. What does immobilization do in addition to permitting the re-establishment of continuity? Another result of immobilization is that the uninjured muscles, tendons and bones in the part are all weakened by atrophy. This has the effect of reducing the gradient between the strength of new bone in the repaired defect and the remainder of the bone. Also the pull of the muscles attached to the bone is reduced. By weakening the whole structure, the strength of the new bone is brought within physiological limits in relation to the functional forces. While all the structures are weakened the re-establishment of the maintenance system permits a gradual strengthening of the part by the same process as structure is continually being adapted to varying amounts of function. By a gradual increase in function the original state is reproduced.

To put the matter in a nutshell, to restore the automatic maintenance system our treatment of a fracture should accomplish two things:

- 1) to permit the re-establishment of continuity,
- 2) to more closely approach balance by weakening the uninjured tissue.

Slide 56. Let us apply this to this ankle situation. Before the accident the structures are in effect maintained in space by the functional forces, as was the case in the dental joint. There is the required balance between the opposing tendons. This balance is destroyed by the accident. Slide 57. If immobilized continuity is re-established, by the process sometimes called fibroplasia. Even when continuity is re-established the repaired tendon is much weaker than its opponent. The repaired tendon must be strengthened but this can only be done by stress, within physiological limits, which is brought about by weakening the opposing structures, and atrophy of muscles. When sufficient weakening of the remaining structure is accomplished the whole structure can be built up by gradually increasing function.

The next question the clinician would ask is now long do these processes take? My guess is that it probably takes about the same time as required for fracture immobilization.

That is only a guess, but let us consider it. According to Howes, in fibroplasia proliferation by the mesenchymal cells through stimuli produced by the wound (pathological) begins 3 or 4 days after wounding; after the inflammatory and exudative stages of healing. The process of fibroplasia can be seen grossly by the 4th day. It is most active in the next 7 to 9 days. During this time there is a deposition of fibrils of collagen between the cells. The fibrils are oriented around the new vessels and with the passage of time their concentration becomes more dense. After 2 weeks of healing they are still of fine size. In the rabbit, 9% of the original strength is restored, in the cat 2% and in the dog 5%. In general less of the original strength is restored in the larger animals in this period. When continuity is established, that is, the wound is completely filled with cells, the pathological stimuli subside and the stress stimuli take over, for functional adaptation of the newly formed connective tissue. This adaptation is a protracted process, taking probably somewhere around 75 days before the tendon regains its original size and strength. This phase rather than the establishment of continuity is probably the greater reason for the slow healing of tendons. So as a guess I'd say in this case the ankle should be immobilized 6 - 8 weeks. Perhaps less time is required with a joint where the stress is not so great as it is in an ankle or knee. However, the time factor could be worked out more accurately by animal experimentation. In general it may be said that the greater the strength of the tendon, the longer it takes for the functional adaptation of the tissue. I have referred to uncomplicated healing. Such things as vitamin C deficiency, infection, cortisone, uncontrolled diabetes, interfere with fibroplasia and the time would be prolonged.

It might be interesting to speculate what might occur if the ankle were immobilized say for only 3 weeks, if balance of forces was not re-established. We know the result of imbalance in our dental joints causes structural changes throughout the whole joint. However, I am not well enough acquainted with ankle or knee joints to predict the possible result.

I have often wondered about the efficacy of immobilizing a joint for the required period where previous treatment had not resulted in a normal joint; giving nature a second chance, so to speak.

As I stated before, one objective of this work is to improve the rationale underlying treatment. To facilitate this use of the concept by the clinician, this work should be organized and summarized. I have also tried to bring out another objective, that is to clarify this complex field in order to more clearly see the problems on which further research is needed. To work with an orthopedic surgeon who is trained in the technical procedures in the surgery involving the bone and joints of the leg would be a great help in speeding up the research.

If this work is of interest to fields other than dentistry, I shall be very happy. But basically I am a dentist and our problems are my first concern, so I will conclude on a dental note.

CONCLUSIONS

The study of physiology leads to an appreciation of the part the development of this science is having in the progress of medical practice. I have been working towards something similar for dentistry, in other words a physiological basis for dental practice. But the application of physiology as taught to-day to dental practice presents difficulties. It seems to me that one reason may be that dental problems are largely connected with the physiology of the skeletal system and standard texts do not deal with this phase of physiology to any great extent. For example, the study of the physiology of the endocrines would mean more to dentists if the emphasis was on their effect on the skeletal tissues. The same could be said of nerve and muscle physiology, nutrition, and so on. To be more specific, the orthodontist deals with disturbances in the growth and development of the facial skeleton that results in a malposition of the teeth. The mechanism described by which the teeth are kept in function and in correct relation with their opponent during growth and the factors which modify this mechanism are of practical interest to him. The periodontist is more interested in maintenance and repair. The concept provides a basis on which the whole problem of malocclusion with which all phases of dentistry are concerned can be approached. Dentistry is becoming more and more aware of the need for a more biological approach, also of the gap between the biological sciences and practice; perhaps this work may help bridge the gap, also point the way towards further development of the phase of physiology applicable to dental practice.

APPENDIX H

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: An evaluation of the disturbances of the dentin formation in the rat incisor following traumas of various origin.

Grantees: Dr. G. Perreault and Dr. G. Pelletier,
Faculty of Dentistry, University of Montreal

Project No.: D.R. 65 Amount of Grant: \$2,600.00

SUMMARY OF WORK

Previous investigations have established the fact that the normal dentin formation in the rat incisor can be disturbed by factors of various origin.

The disturbance is called the calcio-traumatic reaction.

In the present project, we have studied the effect of a number of stressing agents and conditions in order to establish their potentiality to induce this reaction.

Each step of the operative procedure must be tested as contributing some influence in the production of the calcio-traumatic reaction. Experiments were designed to appreciate the effects of some factors as general anesthesia (ether, nembutal) local traumas (bone fracture, cavity preparations with or without drugs, pouch granulomas) and systemic alterations (fast, heat).

The study of the available specimens seems to point towards the following sequence: (in the order of increasing severity) saline, nembutal, fracture, ether, fast, heat, pouch (croton oil), pouch (formalin), sham, open cavity, silver nitrate.

PROGRESS REPORT

INTRODUCTION

A number of investigators have observed that stressing conditions either to humans (5) or animals (1,2,3,4,6,7,8,) were followed by reactions in the pulp and growing dentin of the teeth.

This reaction revealed itself under the form of a calcio-traumatic band, i.e. a sharp reaction of the odontoblasts which may be described in three steps inside the daily incremental line: first, a zone of hypercalcification into the newly formed dentin; second, a zone of hypocalcification underlying the first one, and third, another zone of hypercalcification in contact with the odontoblasts.

This reaction usually takes place in less than twenty-four hours. If the trauma is not very strong, the activity of the odontoblasts is impaired and the dentin formed shows qualitative and quantitative alterations.

PURPOSE OF THE STUDY

The literature reports only the description of reactions to specific traumas but without any attempt to systematization with respect to the intensity of the causative factor or of the reversibility of the tissular reaction. It is our aim to test along with the production of the calcio-traumatic reaction, treatment procedures or the use of agents susceptible to alter the morphological aspect of the dental tissues.

Certain operative procedures involve a series of steps which might be listed as follows:

- First: injection of anesthetics
- Second: incision in the gingiva to uncover the bone
- Third: drilling of the cavity in the bone to uncover the periodontal membrane
- Fourth: opening of the periodontal membrane
- Fifth: cavity preparation in the tooth
- Sixth: sealing of filling or medicament in the tooth.

In the present study we are evaluating the reactions involved in the forementioned steps and also in some

typical stresses such as heat, fast, and fracture. These stresses may be considered as conditions susceptible to disturb the normal equilibrium of the functioning tissue.

EXPERIMENTS:

Table I shows the list of procedures to be indicated as causative factors in the production of the calcio-traumatic reaction. Here are some more details about the operative procedure.

(a) Operative procedure:

1.- Six rats received an intraperitoneal injection of nembutal, 6 mg/ 100 gr of a .6% solution.

2.- Six rats have been left under ether anesthesia of fifteen minutes (open technique).

3.- Five rats received intraperitoneal injection of saline (2 cc/100 gr) without anesthesia.

4.- Six rats were anesthetized with an injection of nembutal. An incision was made in the gingiva to expose the bone. A cavity was made in the bone. The tooth was not touched. A thin layer of paraffin was flowed on the bottom of the cavity to prevent ankylosis and the cavity in the bone was closed with a bland material in the occurrence zinc oxide and eugenol. No suture was made to close the cut in the gingiva.

5.- Six rats were treated as in No. 4, but the operation was carried one step further. A cavity was prepared in the tooth. This cavity was left open for a period of 6 to 7 days.

6.- Same operation as in No. 5 (six rats). This time silver nitrate was applied for ten minutes in the tooth cavity. After that period of time the tooth cavity was wiped dry and filled with zinc oxide and eugenol. Paraffin was flowed over the filling. The bone was closed with zinc oxide and eugenol.

7.- Six animals received ether anesthesia and had their right femur uncovered and fractured. The incision was sutured.

8.- Six animals were subjected to a three days fast and put back to diet for four more days.

9.- Five animals were subjected to heat. Two hours at 40°C.

10.- Other animals were subjected to intradermal irritation. Under ether anesthesia a pouch was produced on the back of the animals by injecting 25 cc of air. In five animals 1 cc of a 2% solution of formalin was injected into the pouch. In five animals 1 cc of 1% solution of croton oil was injected into the pouch.

11.- Six normal unoperated animals served as controls.

The animals were sacrificed after six to eight days or after eleven to sixteen days.

(b) Histologic procedure:

Immediately after sacrifice the mandibles were dissected and fixed in 10% neutral formalin. The specimens were decalcified in 5% nitric acid and embedded in paraffin. Histologic sections were made at eight to twelve microns.

All sections were stained with hematoxylin and eosin.

The criteria used in the evaluation of the dentinal and pulpal reactions are the same as those established by Perreault et al (9). They are summarized in Table II.

FINDINGS

The preliminary results are included in Table III. The stresses are listed in the order of increasingly injurious effects on the functions of the odontoblasts and the pulp.

The effects are presented in terms of:

- 1.- the acute effect of the odontoblasts was reflected in the character of the calcio-traumatic reaction and
- 2.- the recovery of these cells as seen in the quality and quantity of the dentin produced by these cells during the postoperative period.

1 - Injection of saline or nembutal:

The calcio-traumatic reaction in this group of specimens is represented by a slightly accentuated incremental

line. Meaning that the shock to the odontoblasts was very short in duration and low in intensity. The quantity and quality of the postoperative dentin being normal signifies that the recovery has been uneventful.

2 - Fractures, Ether anesthesia

These two series are assessed together because they present almost identical histologic images. The calcio-traumatic reaction is more pronounced and the incremental lines of the postoperative dentin are accentuated. This represents a more prominent

3 - Fast

In this series the immediate reaction is slightly accentuated (1). While the postoperative dentin appears quite normal, incremental lines are missing in at least two specimens. The missing incremental lines would correspond to the number of days of fast. What causes the lines not to be formed is not quite sure. But it has been previously observed (9) that after a controlled survival time of seven days where seven incremental lines should have formed only six were found in a number of animals. It was tentatively explained that failure to eat after an operation could be the cause.

4 - Heat, Intradermal irritation, Sham operation

The histologic picture of these specimens are quite alike. While the calcio-traumatic lines in the sham operation and intradermal irritation are definitely more accentuated the same reaction in the specimens subjected to heat is less. But, while the incremental lines in the postoperative dentin of the specimens subjected to sham and intradermal irritation has a strong tendency towards accentuation the specimens subjected to heat presents a broader variation in postoperative dentin going from normal to disturbance in calcification represented by interglobular dentin. This would mean that in the second and third operations the reaction has been more acute but the recovery while affected somewhat was quite good. In the first operation the reaction while being less severe the recovery has been slower and not without some trouble.

5 - Open cavity

The immediate reaction is accentuated. But the most interesting observation is that as the cavity gets

deeper the quality and quantity of the postoperative dentin change. When the cavity is a shallow one the postoperative dentin is normal. If the cavity is of medium depth the recovery of the cells is more difficult. If the cavity is very deep (less than twenty microns from the body of the odontoblasts), we get hypoplasia confined to the postoperative dentin under the cavity. This means that the odontoblasts react inversely to the amount of shock. If the shock is very strong, the cells have less chance to recover. The fact that the cavity is left opened has probably not very much effect because very soon the connective tissue of the periodontal membrane grows again and covers the cavity, protecting it from external irritants such as saliva.

6 - Silver nitrate

The same phenomenon as in the previous group is observed here, but silver nitrate adds an enormous load that the odontoblasts cannot, most of the time, overcome. The initial reaction is very much accentuated and the postoperative dentin is always markedly deficient either in amount or in quality.

In even shallow cavities hypoplasia is observed. In very deep cavities aplasia occurs and most of the circum-pulpal postoperative dentin is strongly hypoplastic.

This means that not only the odontoblasts under the cavity are killed but that the other cells are reduced in their potency. It also means that silver nitrate affects the pulp itself and consequently the connective tissue from which the odontoblasts come into being.

How the silver nitrate penetrates through the dentin is not quite clear; but the fact is sure that it penetrates and that the penetration continues after the cavity has been washed and dried.

SUMMARY AND CONCLUSION

The present investigation was designed to study the effect of traumatism on the constantly growing pulp and dentin of albino rat incisors.

A comparative study of the effects of these traumas is presented.

Table I : Groups of animals classified as per treatment or operative procedure.

1. injection of nembutal
2. ether anesthesia
3. injection of saline solution
4. sham
5. open cavity
6. application of silver nitrate to open cavity
7. fracture
8. fast
9. heat
10. intradermal irritation with formalin or croton oil
(pouch technique)
11. controls.

Table II : Explanation of the criteria used to evaluate the effects of stresses on the functions of the odontoblasts.

Dentin	Calcio-traumatic reaction	N. (normal)	A slight accentuation of incremental line formed at time of the operation (calcio-traumatic band) surrounds the pulp completely.	
		1.	Calcio-traumatic band slightly more prominent.	
		2.	Calcio-traumatic band significantly more prominent.	
		3.	Hypocalcified zone of calcio-traumatic band very wide. Represents longer arrest in calcification of dentin and indicates a moderately severe reaction of odontoblasts.	
		4.	Dentinal tubules interrupted. Represents severe acute injury of odontoblasts.	
Postoperative dentin	Quality	N,	Indicates that calcification, structure & amt. of dentin formed postoperatively was same as in controls.	
		I,	Definite disturbance in postoperative calcification of dentin. Characterized by accentuation of incremental pattern.	
		2,	Moderately severe disturbance in calcification including interglobular dentin. Indication of slow recovery by odontoblasts from acute injury produced by stresses or traumas.	
	Calcification	3,	Dentinal tubules reduced in number.	
		4,	Dentin matrix is tubules, less homogenous & fibrillar. Dentin usually hypoplastic.	
	Structure	N,	Amt. of dentin formed normal compared to controls.	
		Hypoplasia,	Dentin deficient in amt. Severe injury to odontoblasts, partial recovery of cells.	
	Quantity	Aplasia,	No postoperative dentin formation. Death of all odontoblasts.	

Table III : Summary of effects of trauma on the pulp, the odontoblasts and the dentin of albino rat incisors. (See Table II for detailed explanation of symbols)

Time elapsed between treatment & sacrifice	Procedure	Calcio-traumatic reaction	Postoperative calcification & structure	Effects on odontoblastic functions
39 days	Controls		N	
6 days	Saline solution	N	N	Transitory with fast, complete recovery
6 days	Nembutal	N	N	
6-8 days	Fracture	I	N	Initial reaction more marked. Recovery complete
6 days	Ether	N-I	N-I	
7 days	Fast	I	N	Seems transitory but in some cases no incremental lines (corresponding to fast) are found
7 days	Heat	I	N-2	Transitory with complete recovery in day or two. In case of heat recovery was slower
16 days	Pouch (croton oil)	I-2	N-I	
11 days	Pouch (formalin)	I-2	N-I	
6-7 days	Sham	I-2	N-I	
6-7 days	Open cavity	I-2	N, shallow cavity. I, medium cavity. Hypoplasia (small) in deep cavities	Transitory to permanent injury, depending on depth of cavities
6-7 days	Silver nitrate	2-4 according to depth of cavity	Hypoplasia to aplasia according to depth	Destructive. Hypoplasia in shallow cavities. Aplasia elsewhere

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APPENDIX I

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.

Grantee: Dr. C. P. Leblond,
Dept. of Anatomy, McGill University

Project No.: D. R. 23 Amount of Grant: \$3,500.00

SUMMARY OF WORK

Two lines of investigation are reported:

(1) The entry of labelled glucose into the organic matrix of teeth is described in a final report on the subject. In enamel, the radioactive label of glucose is taken up in the organic material making up the layer or preenamel. Later, the radioactivity is seen within the maturing enamel matrix, but its amount decreases. Within a week, the radioactivity becomes undetectable. The available evidence indicates that the labelled hexose has been taken up directly into a carbohydrate of enamel. In dentin, the radioactive label of glucose is taken up within predentin and, with the passage of time, is seen in dentin proper, but its amount does not change with time. Even after a 6 month period, the dentin radioautograph is as intense as 24 hours after radioglucose injection. The available evidence indicates that products of glucose metabolism rather than glucose itself are taken up in the collagen of newly-formed dentin. This material remains stable indefinitely.

(2) Preliminary chemical analyses of enamel are also reported.

1. VISUALIZATION OF C^{14} IN THE TOOTH MATRIX AFTER INJECTION OF LABELLED HEXOSES (with Dr. J. E. Leach)

Carbohydrates of high molecular weight have been demonstrated in the matrix of dentin (Pincus, 1943, 1947; Rogers, 1941; Black, 1951; Hess and Lee, 1952; Kunita, 1953) and enamel

PROGRESS REPORT

The work carried out by our group in the past consisted in administering labelled substances presumed to be precursors of either the mineral or the organic components of the tooth, and in tracing the site and rate of their incorporation by means of parallel radioautographic and chemical analyses. Thus, P^{32} -phosphate and calcium⁴⁵ have been injected to investigate the deposition of minerals in the growing tooth, while C^{14} -bicarbonate has been used to examine the formation of the dentinal matrix. This work has been summarized in an article including also the work of Drs. Belanger and Greulich (Ann. N.Y. Acad. Sc. 1955, 60, 629-659).

More recently, in the hope of achieving a more searching analysis, two types of investigations were carried out:

1) Use of more complex precursors than those listed above. In the course of this year, C^{14} -glucose and S^{35} -methionine were used to investigate the formation of carbohydrates and proteins respectively. Dr. Kumamoto and the writer have already reported on work done in animals injected with radioglucose. The present report describes how this work has been amplified. Conclusions of importance with regard to the stability of the dentinal matrix and connective tissue in general have been reached and are nearly ready for publication. (The need for supplementary technical assistance arose during completion of this investigation. Additional support from a grant of the Defence Research Board was required and will be acknowledged in the publication.)

2) Additional chemical analyses of enamel were undertaken. Except perhaps for recent work by Stack, M.V. (Ann. N.Y. Acad. Sc. 1955, 60, 585), it was felt that the analyses of enamel found in the literature were not sufficient to allow us to explain our radioautographic observations. Chemical analyses of a series of fractions of enamel matrix were therefore carried out by Dr. H. Vance and Miss I. Karpishka.

I. VISUALIZATION OF C^{14} IN THE TOOTH MATRIX AFTER ADMINISTRATION OF LABELLED HEXOSES (with Dr. Y. Kumamoto).

Carbohydrates of high molecular weight have been detected in the matrix of dentin (Pincus, 1948, 1952; Rogers, 1949; Stack, 1951; Hess and Lee, 1952; Kumamoto, 1955) and enamel

(Pincus, 1949; Nikiforuk, 1956), but no information is available regarding the mode and site of their production. Recent reports have shown that labelled glucose is a precursor of certain carbohydrates, particularly hyaluronic acid and chondroitin sulfuric acid (Roseman et al, 1953; Dorfman, 1954; Schiller et al, 1956). Glucose might also be a precursor of the carbohydrates appearing in dentin and enamel. Therefore, radioautographic studies of teeth after administration of labelled glucose should provide useful information on the formation of carbohydrates in this structure. Accordingly, C^{14} -containing glucose was injected into young rats and at various time intervals thereafter, their teeth were examined radioautographically. Parallel experiments were conducted with C^{14} -mannose.

Materials and Methods

In all experiments, 3 day old rats were given labelled hexoses obtained from the Radiochemical Centre, Amersham, England*. After a period varying from three hours to several months after administration, the animals were sacrificed by chloroform or ether. Upper and lower jaws were excised and fixed in a solution of 3 parts of 95% alcohol to 1 part of commercial formalin neutralized with potassium hydroxide. Tissues removed 14 days or more after administration were decalcified in sequestrene (ethylene-diaminetetrasodium acetate). Histological sections, either unstained or stained with hematoxylin-eosin or periodic acid (PA)-Schiff were coated with Kodak NTB3 emulsion, dried and exposed in the dark. After a period of exposure varying from 3.5 - 7 months the sections were processed through developer and fixer, dehydrated in alcohol and xylol, and mounted in Canada balsam or Permout.

Radioglucose, uniformly labelled with C^{14} , was administered in 3 series of experiments in a dose of 30 μ c per animal. In the first series, 2 animals received an injection by the intragastric, and 2 by the subcutaneous route; one animal of each pair was sacrificed at 3 hours and the other, at 24 hours after the injection. In the second series, three animals were injected subcutaneously and sacrificed at 3, 24 and 72 hours respectively. In

* Pilot experiments carried out with labelled glucose kindly supplied by Dr. G. Krotkov, Queen's University, Kingston, Ontario, had shown the interest of this work.

the third series, 18 animals were injected intragastrically and two of them were sacrificed at each of the following times: 3 hours, 1, 3, 6, 14 days, 1, 2, 4 and 6 months.

Radiomannose was administered to three animals, each one of which received 23 μ c of mannose- C^{14} subcutaneously. They were sacrificed at 3, 24 and 72 hours later respectively.

Results

Whether radioglucose was administered intragastrically or subcutaneously, the distribution of C^{14} was the same in the teeth; and therefore the following description of the radioautographic reactions applies to both routes of administration.

The developing molar tooth examined at the 3 hour interval (Fig. 1) showed two dark lines of reaction. One of them extending along the whole inner surface of the three cusps was associated with the dentinal organ. The other, an outer line which appeared discontinuous and was particularly developed on the occlusal portion of each of the three cusps, was associated with the enamel organ. The incisor tooth exhibited two comparable reaction bands, a circumpulpal one in relation to the dentin, and a second one on the convex surface where enamel develops.

Examination of the first molar and incisor teeth at a higher magnification revealed that the inner band of reaction emanated from predentin and in fact could be assigned to that portion of predentin adjacent to the odontoblasts lining the pulp cavity (Fig. 10, arrow slanting upwards). The outer band of reaction was limited to the portion of the enamel organ known as preenamel, that is, an area approximating the taller ameloblasts (Fig. 10, arrow slanting downwards).

At the second interval, 24 hours after administration, both reaction bands were still clearly distinguishable around the first molar (Fig. 2) and incisor teeth. The inner band was increased in intensity (Fig. 11), but the outer band was fading, particularly in the case of the incisor. The site of the inner band was no longer predentin, but the innermost portion of the dentin proper. The outer band, now absent from preenamel, appeared in the middle region of the enamel organ. It was less precisely limited than at the first interval (Fig. 11).

At the three-day interval the inner band which by now occupied the midportion of dentin persisted without detectable change in intensity at the same distance from the dentino-enamel junction as at the previous interval (Figs. 3 and 12), whereas the enamel band appeared broader and fuzzier than at earlier time intervals (Fig. 3) and, under high magnification, was represented only by a few scattered silver grains (Fig. 12).

At the 6-day interval (Figs. 4 and 13) no significant enamel reaction was visible, while at 14 days and later, it was not possible to observe the enamel, which had been eliminated by decalcification. At the 6- and 14-day intervals (Figs. 13 and 14), the dentinal reaction did not change in intensity, nor in its distance from the dentino-enamel junction; but, owing to the continuous accumulation of layers of new dentin on the inside, the distance between the band of reaction and the pulp increased gradually.

By the 14th day the molar teeth were about to erupt; thus their occlusal surface was intact. However, immediately after their eruption into the oral cavity, their surface became worn away by attrition due to grinding against food or opposing teeth. Accordingly, during subsequent intervals (1-6 months) the height of the crown decreased progressively as may be seen in Figs. 6-9. At the one month interval, only the superficial portion of the cusps had been eroded and, as a result, the portion of the reaction band present at the apex of the cusps was lost (Fig. 6). The distance from the band to the dentino-enamel junction could still be measured (at the top of picture in Fig. 15) and was approximately the same as at the 14-day interval (Fig. 14) and earlier. Meanwhile, the dentin had been continuously increasing in thickness as the odontoblasts receded and thus the initial pulp cavity of the cusps gradually decreased in size (compare Figs. 4, 5 and 6) and was eventually obliterated, as in Fig. 7. The open pulp cavity thus seemed to withdraw gradually below the cusp, as shown by the increase in the distance from the occlusal surface (compare Figs. 14 and 15). At the 2-month and later intervals the cusps gradually wore away (Figs. 7-9), but the dentinal reaction was seen in their lower portion.

Since the radioautographs obtained at the 1, 2, 4 and 6 month intervals had been exposed and developed simultaneously, they could be compared to one another. Such comparison showed that the intensity of the band reactions did

not change with time (Figs. 15-18). Neither did their relation to the dentino-enamel junction appear to be modified.

The results obtained after administration of radio-mannose were, in all respects, similar to those obtained with glucose. Thus, under low power, the pictures showed the persistence with time of the dentinal band, and the rapid fading of the enamel band reaction. At the higher magnification, the intensity of the dentinal band was seen to increase from the three to the 24 hour interval without change occurring afterwards. In contrast, the enamel reaction was maximal at 3 hours and later became more diffuse and less intense.

Examination of tissues other than dentin and enamel revealed a widespread but similar distribution of radioactivity after radioglucose or-mannose administration. Of the tissues associated with the tooth, the stellate reticulum (Fig. 1, S) gave a slight reaction which decreased with time (Fig. 3, S). The ameloblast layer also gave a definite reaction (Fig. 1, A), with a maximum in those areas of primitive tooth buds where new ameloblasts arise by mitosis (YA Fig. 1). With time, the ameloblastic reaction decreased and disappeared, but not in the zones corresponding to the more intensely reactive ameloblasts, in which a reaction was recognized at 14 days (Fig. 19, A) and at 1 month. At those times, the reaction was restricted to the nuclei of some ameloblasts and of rare cells in the stratum intermedium (Fig. 19 S).

The pulp gave a slight reaction (Fig. 1, P) especially in the more primitive bud (Fig. 2, P), and the reaction decreased with time. The odontoblasts in contrast gave no significant reaction, except again in the areas where they form by mitosis (Fig. 1, YO). This particular reaction did not decrease with time (Fig. 20) and was in fact recognized at all time intervals, even at 6 months, in some odontoblasts towards the base of the crown in the second molar. While the cytological location of the reaction was not definitely established, radioactivity seemed to be present in the nucleus and perhaps also in cytoplasmic or paracytoplasmic material.

Alveolar bone reactions were seen (Fig. 1, B). These and other bony structures showed a maximal reactivity in areas of new bone formation, which persisted (Fig. 3, B) until removed by processes of resorption. In long bones,

the labelled material was taken up on the outer surface mainly, was found within the matrix by the third day, was made to approach the endosteal surface which was being eroded away, and finally was itself eroded and disappeared. By 1-6 months almost all bony reactions had thus been removed; only rare, small areas were found to escape resorption and persist throughout the experimental period.

Other tissues at 3 and 24 hours displayed intense reactions but gradually lost their radioactivity. After the 6 day interval, tissues were unreactive in preparations exposed for 3-5 months; and it was necessary to counterstain the sections in order to photograph the dental reactions (Figs. 4-9). However, radioautographs exposed for 7 months revealed two types of weak reactions, namely, 1) over a few scattered nuclei, particularly those in the outer nuclear layer of the retina, and 2) over the collagenous fibers in a few connective tissue areas, particularly in the lamina propria of esophagus and of bladder. Other soft tissues were unreactive.

Discussion

Chemical nature of the radiocarbon taken up in hard tissues

The radioautographic reactions observed in hard tissues after administration of C^{14} -glucose were not affected by the acid medium during H & E staining or during decalcification. Therefore, the labelled material observed in the radioautographs was not in a mineral but in an organic form. Since radiocarbon was retained during histological procedure, it may be presumed not to consist of soluble substances, such as the free hexoses themselves or their metabolic products (pyruvate, bicarbonate, etc.), but rather to be taken up into substances of high molecular weight, such as the protein, collagen, and the small amounts of various carbohydrates found in dentin. A priori, it was not possible to state whether hexoses were taken up as such in these large molecules or were first broken down into metabolites (e.g. bicarbonate) which were then synthesized into the large molecules.

A partial answer was supplied by a comparison with radioautographs obtained at various time intervals after administration of C^{14} -bicarbonate (Greulich and Leblond, 1954). In dentin, the pattern observed under these conditions was indistinguishable from that described above for C^{14} -glucose. In enamel, however, only a negligible reaction

was produced with C^{14} -bicarbonate in contrast to the intense one arising after administration of C^{14} -glucose (Figs. 1-3).

These, and similar observations carried out in soft tissues (Kumamoto, unpublished) indicated that the reactions observed with labelled bicarbonate were also present with labelled glucose. The reverse was not true, since some reactions such as that of the enamel (and also goblet cells) were seen only after glucose administration.

Since the enamel reaction was observed only after radioglucose administration, it must have been due to the uptake of glucose itself or of a glucose metabolite which is not readily synthesized from bicarbonate. Nikiforuk (1956) has demonstrated the presence of carbohydrates in the enamel. Should the finding of Schiller and collaborators (1956) that glucose enters the hexosamine and probably the glucuronic acid residues of acid mucopolysaccharides apply to enamel matrix formation, the possibility arises that glucose itself may be taken into the polysaccharide which Nikiforuk believes is present in dentin.

The dentinal reaction, which was similar after labelled glucose or bicarbonate, may be due to the incorporation of a common metabolite of these two substances, which may in fact be bicarbonate itself, since glucose is readily degraded to carbon dioxide (Fruton and Simmonds, 1953). The entry of the C^{14} from glucose into dentin as bicarbonate, or a common metabolite of the two substances is supported by the appearance of the C^{14} label of glucose in predentin (Fig. 10), a region which histochemical tests show to be poor in carbohydrates (Leblond, Belanger and Greulich, 1955), whereas the uptake of glucose as a precursor of carbohydrates would have been likely to occur at the predentino-dentinal junction, where the deposition of S^{35} -sulfate indicates carbohydrate synthesis.

The radioautographic reactions observed after administration of labelled mannose were qualitatively identical to those observed after labelled glucose. This was not unexpected for dentinal reactions, since mannose like glucose may be assumed to degrade to bicarbonate and related metabolites. The similarity of the pattern observed in enamel with the two hexoses was more surprising. Perhaps mannose transforms into glucose. Perhaps enamel utilizes both hexoses indiscriminately for the polysaccharide synthesis that may occur in this tissue.

Conclusions regarding growth of teeth

The radioa lographic pattern observed soon after administration of C^{14} -glucose was characterized by the appearance of bands in enamel (e.g. Fig. 1), and dentin (e.g. Fig. 10). These bands were in fact sections of layers of labelled material, which initially arose in close contact with the apices of ameloblasts in the preenamel and odontoblasts in the predentin. Such observations provide a direct demonstration of the accepted belief that these cells elaborate layers of their respective matrices. Furthermore, since identical observations were made at whatever time of the day the animals had been given C^{14} -glucose, it may be concluded that the addition of material to the matrix proceeds continuously in both tissues.

The accretion of matrix may be expressed in another way. With time, the circulating C^{14} -glucose decreased and was replaced by unlabelled glucose, and, consequently, newly formed matrix also became unlabelled. This process produced eventually a separation of the labelled layer from the matrix producing cells by unlabelled matrix, the thickness of which increased gradually with time (Fig. 1-9).

As the labelled layer was displaced from the matrix producing cells, it underwent the transformations as postulated in the accepted hypotheses. Thus, in the tooth, predentin became converted into dentin, preenamel into young and transitional enamel, and, in so doing, both matrices acquired the ability to calcify (abruptly at the predentino-dentinal junction and gradually in the young and transitional enamel).

Later, the development of the matrices differed markedly. In the enamel organ, reactive material on reaching the zones of calcification, diminished gradually, due either to a true departure of labelled material or perhaps to its dilution by the considerable deposition of minerals taking place. In contrast, the labelled materials laid down in dentin were retained for 6 months after radioglucose administration (Figs. 10-18) and it may be safely assumed that they would persist indefinitely, unless dentin is removed by attrition at the occlusal surface. This persistence of dentinal matrix was all the more striking when compared to the gradual disappearance of the reactions in the majority of the soft tissues.

Besides dentinal matrix, persistent reactions were seen only in a few odontoblasts (Fig. 26), in bone matrix,

and in some nuclei (ameloblasts, Fig. 25; retina). It may be speculated that the available results indicate the indefinite persistence in the body of only two types of structures: 1) nuclei in those cells displaying mitotic activity at the time of glucose injection, e.g. dividing ameloblasts and odontoblasts; perhaps the persistence of the reactions testifies to the stability of desoxyribonucleic acid; 2) dentinal and bone matrix, and several connective tissue areas. Since these three tissues are all rich in collagenous fibers and, in the first two cases at least, the reactive zones were obviously newly formed, it is suggested that these persisting reactions are due to radiocarbon incorporated into collagenous fibers elaborated at the time of radioglucose injection. Thus, only two of the numerous substances arising from glucose metabolism appear to be capable of indefinite stability: desoxyribonucleic acid and collagen. The structures of the tooth illustrate both cases.

A band form of radiocarbon deposition app-
ears in preamel (Fig. 1, E) and predentin
(Fig. 1, D) in the three cups of the first
molar. With time the bands are seen in
enamel and dentin proper, but while the en-
amel band decreases in intensity (Figs. 2
and 3, E), the dentinal band shows no app-
arent change with time.

Other tissues showed a definite reactivity,
which decreased by the third day. Note
reactivity in the pulp (Fig. 2, P), oral
epithelium (Figs. 1 and 2, O), alveolar
bone (Figs. 1 and 2, B), cartilage (Fig. 1,
C), nasal mucous glands (Fig. 1, M).

Figs. 1-3. Unstained coated radioautographs of the developing upper first molar tooth of rats given C^{14} -glucose at 3 days of age. The teeth were not decalcified. Exposure was for 3.5 months. The edge of the second molar is visible on the right hand side. X 21.

Fig. 1. 3-hour interval

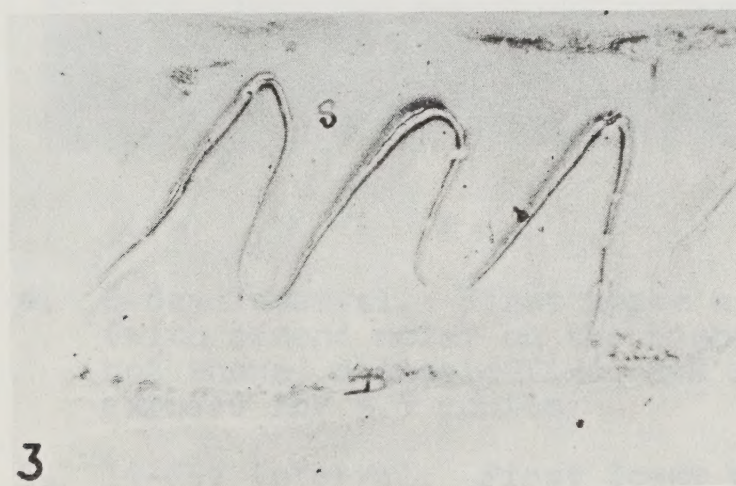
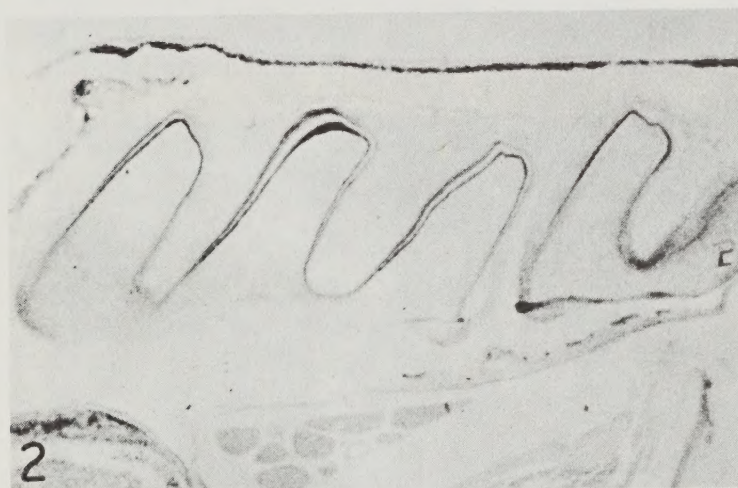
Fig. 2. 1-day interval

Fig. 3. 3-day interval

A band form of radiocarbon deposition appears in preenamel (Fig. 1, E) and predentin (Fig. 1, D) in the three cusps of the first molar. With time the bands are seen in enamel and dentin proper, but while the enamel band decreases in intensity (Figs. 2 and 3, E), the dentinal band shows no apparent change with time.

Other tissues showed a definite reactivity, which decreased by the third day. Note reactions in the pulp (Fig. 2, P), oral epithelium (Figs. 1 and 2, O), alveolar bone (Figs. 1 and 3, B), cartilage (Fig. 1, C), nasal mucous glands (Fig. 1, M).

Besides dentinal matrix, persistent reactions were seen only in a few odontoblasts (Fig. 26), in bone matrix,



Figs. 4-9. Stained coated radioautographs of molar teeth of rats given C^{14} -glucose at 3 days of age. X 21.

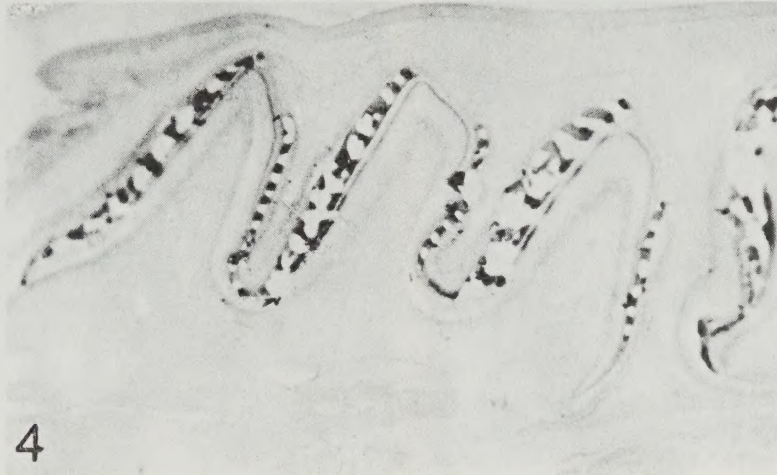


Fig. 4. 6-day interval. First upper molar (with second molar on the right but not shown) undecalcified, H-E stained, exposed for 3.5 months.

Fig. 5. 14-day interval. First lower molar (second molar on the left), decalcified, H-E stained, exposed for 3.5 months.

Fig. 6. 1-month interval. First upper molar (second molar on the left), decalcified, PA-Schiff stained, exposed for 7 months.

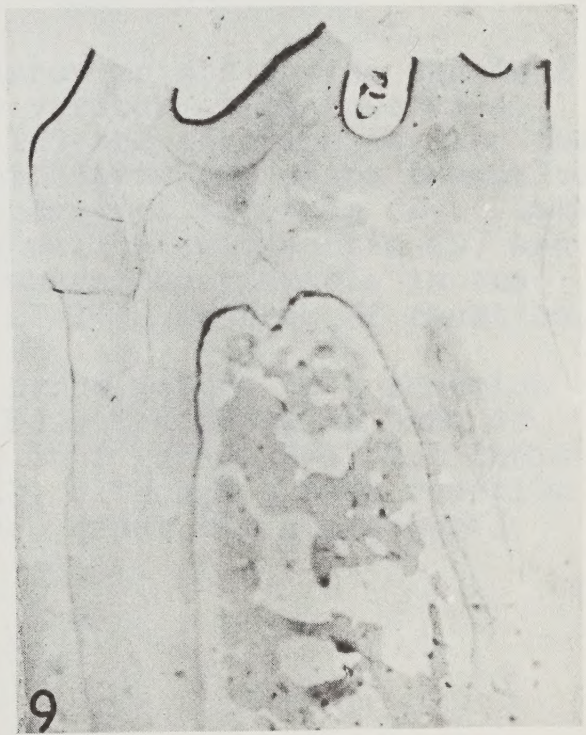
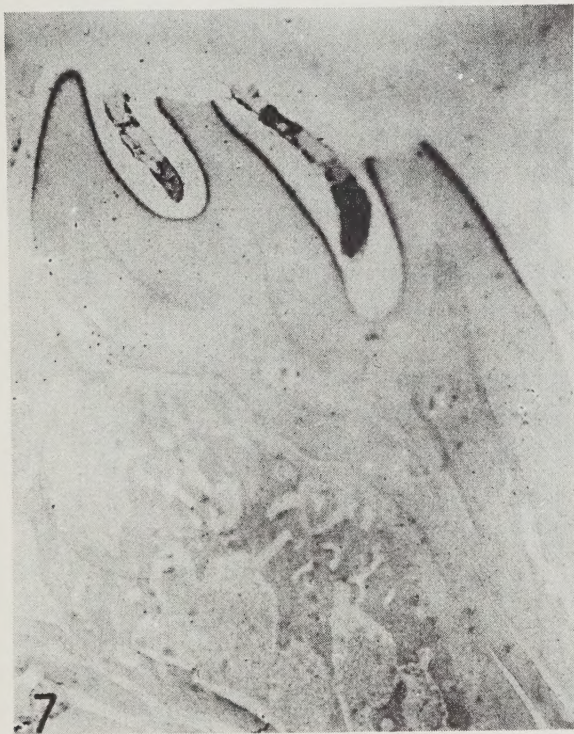
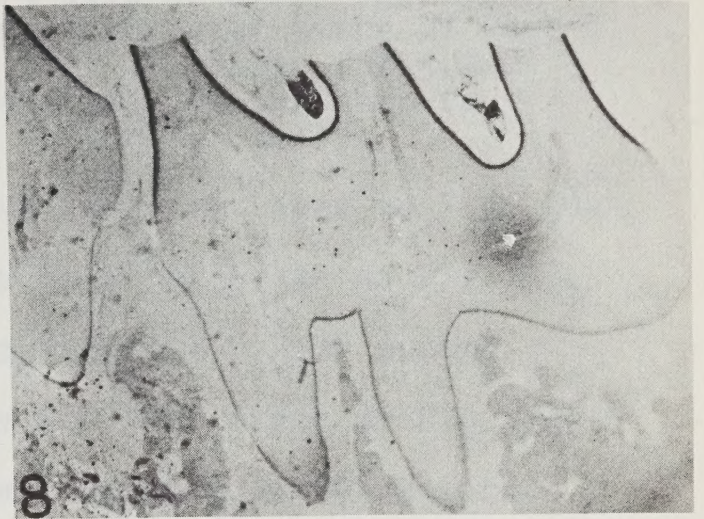
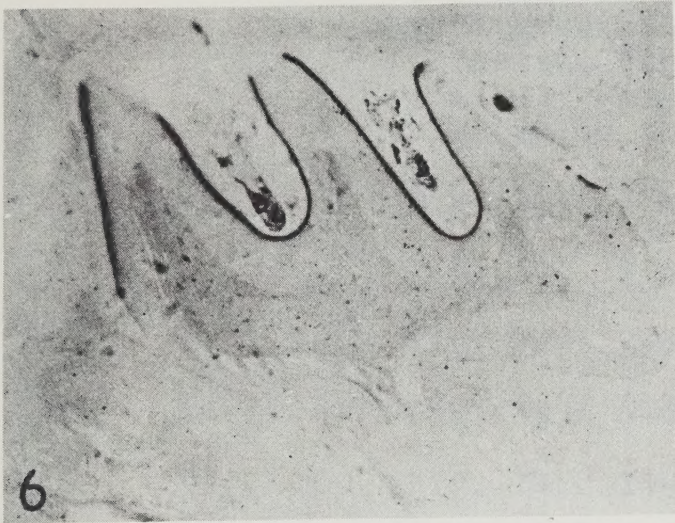


Fig. 7. 2-month interval, same as Fig. 6.

Fig. 8. 4-month interval, same as Fig. 6.

Fig. 9. 6-month interval. First lower molar (second molar on the left), decalcified, PA-Schiff stained, exposed for 7 months.

An intense dentinal reaction (D) persists at all time intervals without apparent change in pattern or intensity. However, as time goes on, the tips of the three cusps are increasingly eroded by attrition.

Figs. 1-14. H-E stained coated radioautographs of cusp; of the first molar of rats given C^{14} -glucose at 3 days of age. The teeth were undecalcified (except at the 14 day interval, Fig. 14). Exposure for 3.1/2 months. Only one side of the cusp is visible in the pictures. X 107.

Fig. 10. 3-hour interval (same as Fig. 1).

Fig. 11. 1-day interval (same as Fig. 2).

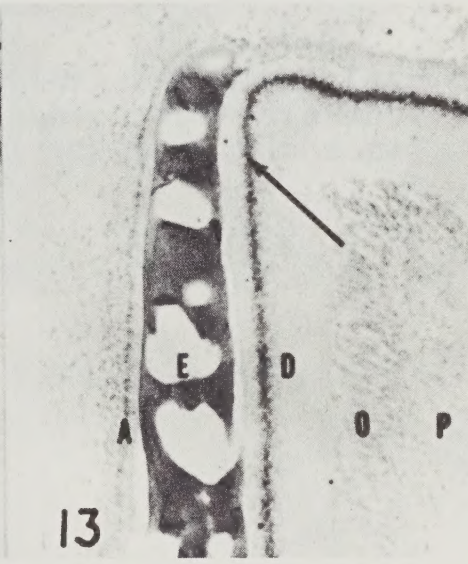
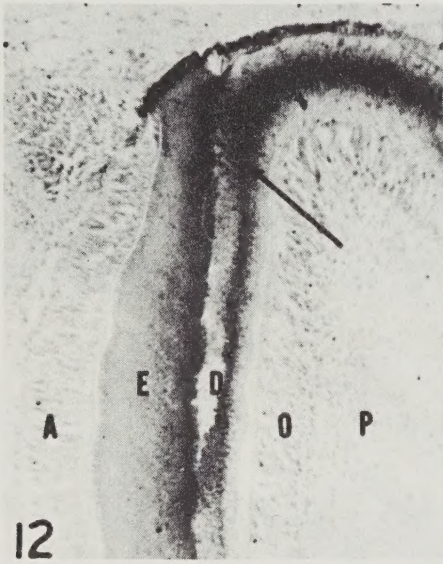
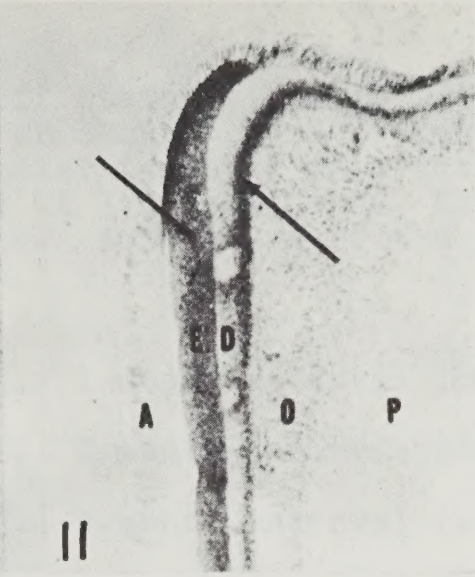
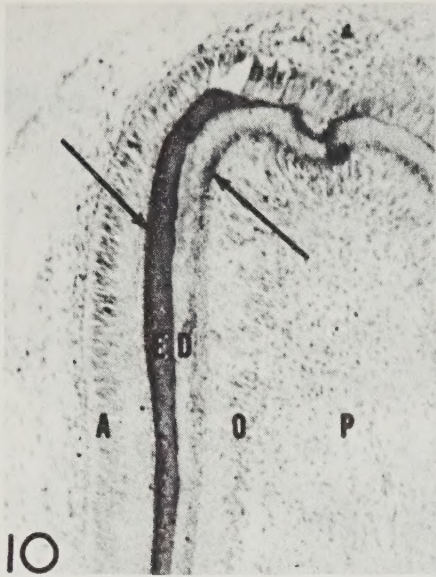
Fig. 12. 3-day interval (lower molar).

Fig. 13. 6-day interval (same as Fig. 4).

Fig. 14. 14-day interval (lower molar).

The ascending arrow points to the dentinal reaction at the various time intervals. At first (Fig. 10) the reaction is near the tip of the odontoblasts (O) which themselves are at the periphery of the pulp (P). Later the reaction is well within dentin (D) and the predentin, where recognizable in the figures (Figs. 11-14), is free of reaction.

The descending arrow points to a reaction in the enamel (E). The reactive band is distinguishable in figures 10-12 but indistinct in Fig. 13, although a weak reaction may still be seen under the microscope.



Figs. 15-18. PA-Schiff stained, coated radioautographs of single cusps of decalcified first molar teeth of rats given C¹⁴-glucose at 3 days of age. Exposure for 7 months. Both sides of the cusps are visible. X 107.

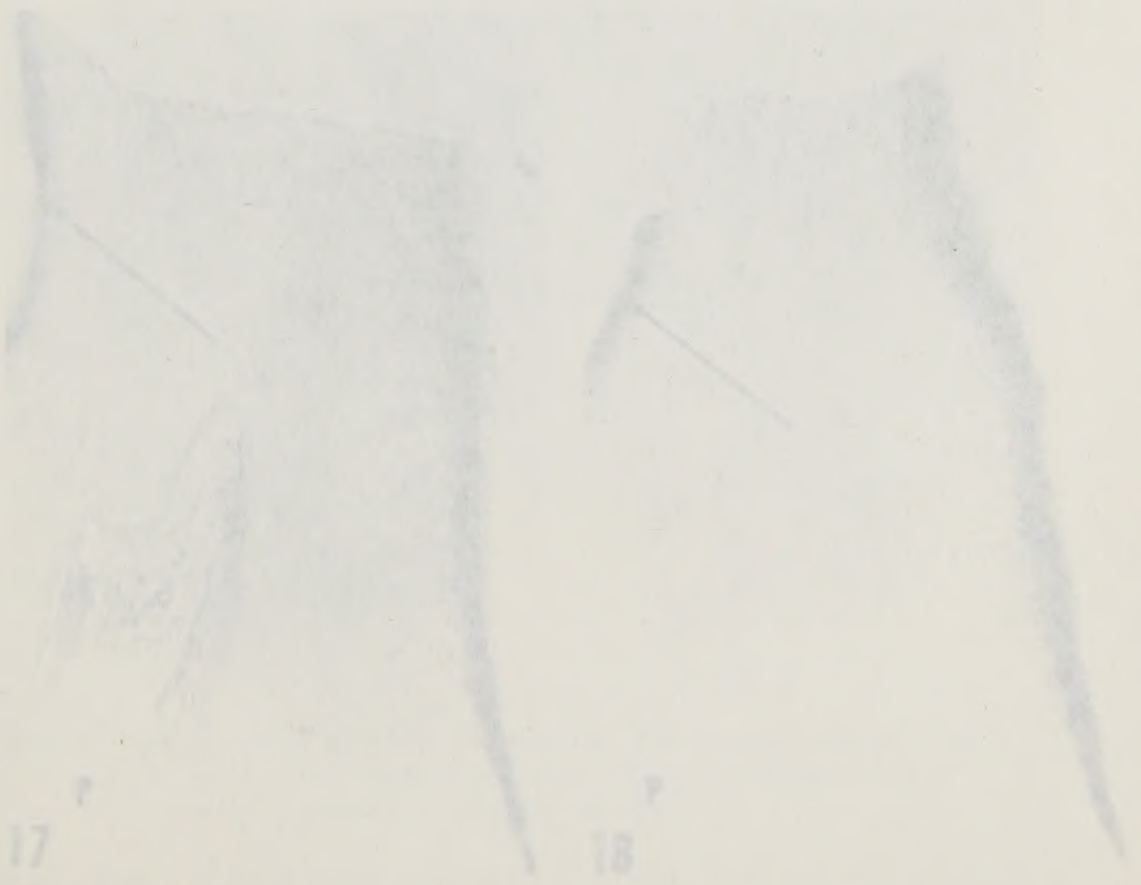
Fig. 15. 1-month interval (Same as Fig. 6)

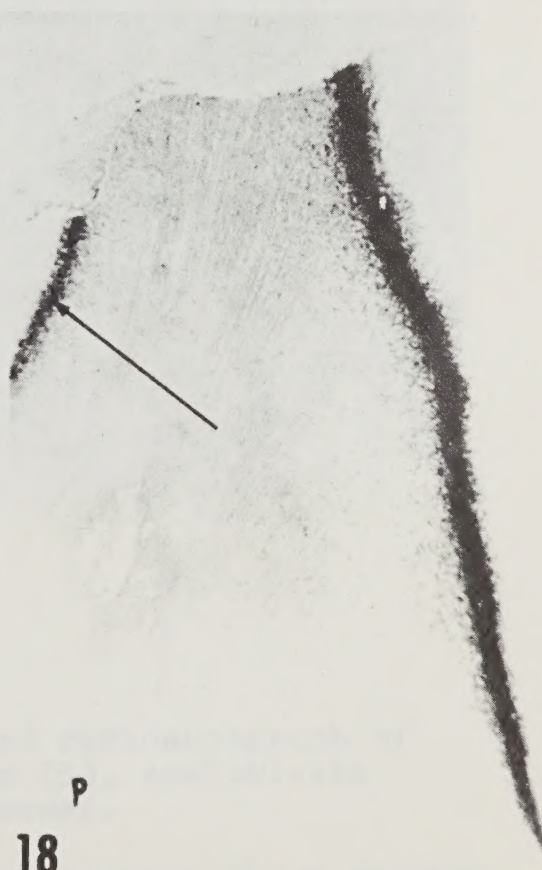
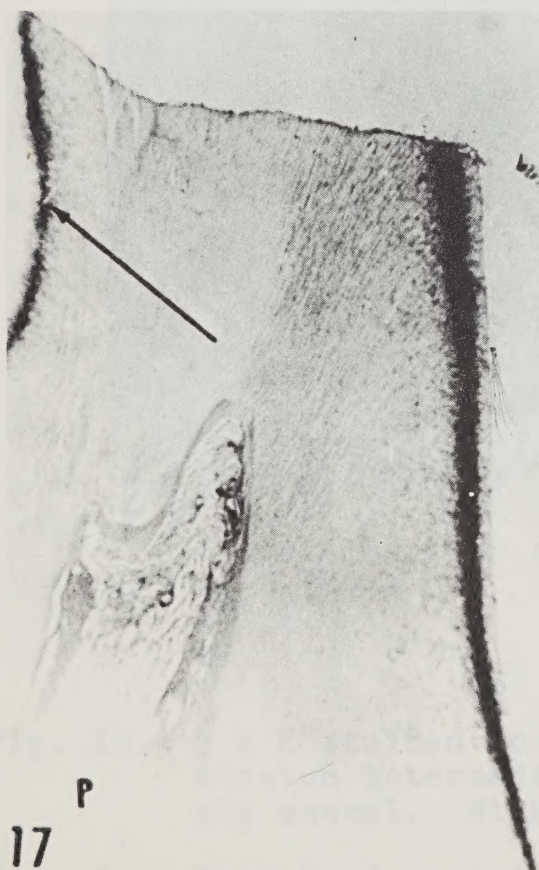
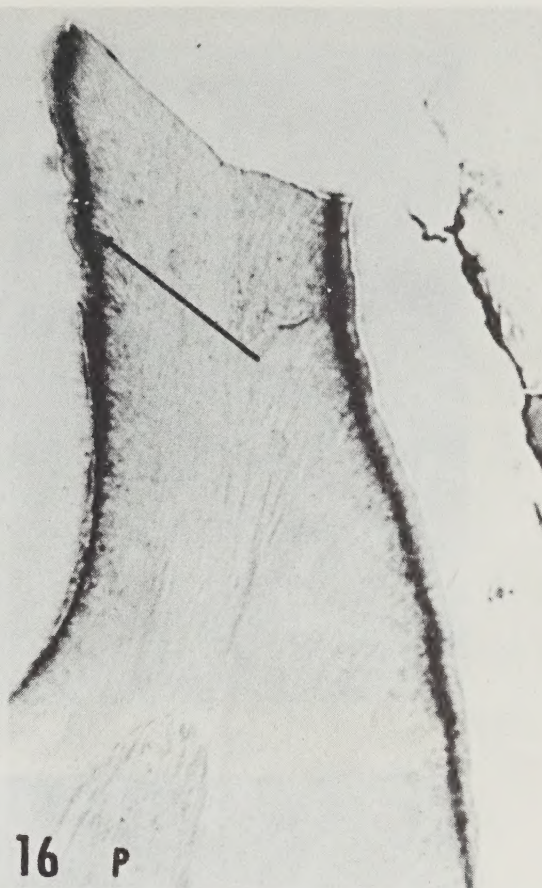
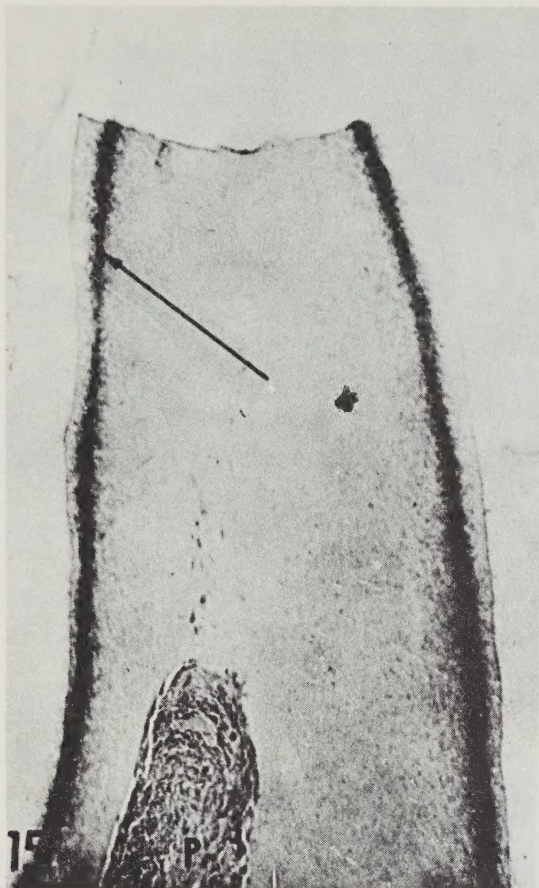
Fig. 16. 2-month interval (Same as Fig. 7).

Fig. 17. 4-month interval (Same as Fig. 8).

Fig. 18. 6-month interval (Same as Fig. 9).

The dentinal reactions (ascending arrows) are intense. Note distance from the occlusal surface to the pulp.





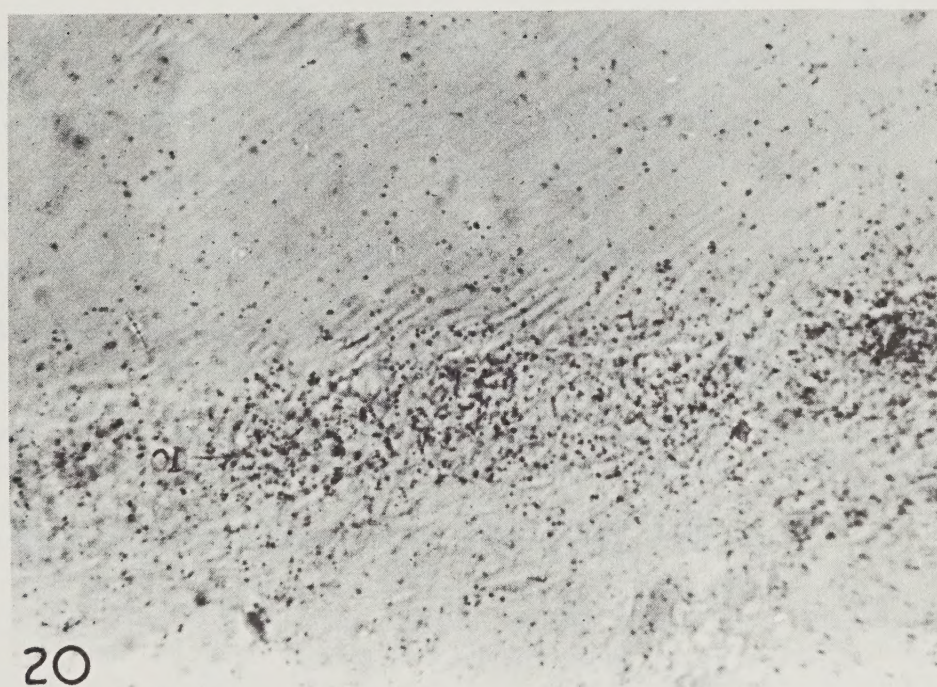
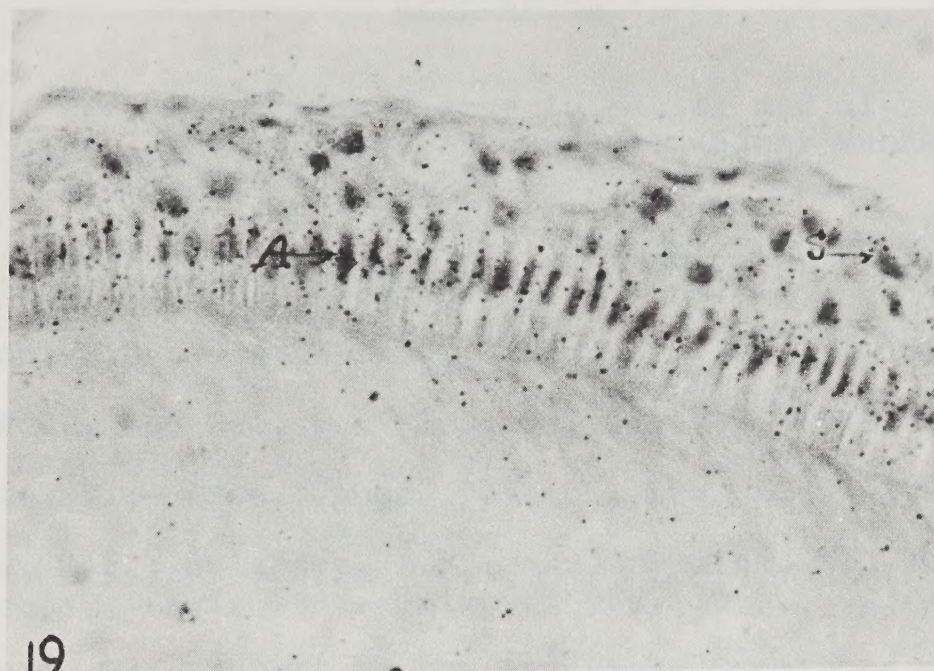


Fig. 19. H & E stained coated radioautograph of stratum intermedium (S), ameloblasts and enamel. High power.

Fig. 20. Unstained coated radioautograph of dentin, odontoblast and pulp (OD). High power.

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II. INVESTIGATION OF THE ORGANIC COMPONENTS OF ENAMEL MATRIX (with Dr. H. Vance and Miss I. Karpishka)

The present investigation was undertaken to gain further insight into the physical and chemical properties of the organic matrix of enamel. Much of the work reported in the literature has centered about the nature of the protein components of enamel. Losee and Hess (1949), Losee, Hess and Neidig (1950), Stack (1955), Battistone and Burnett (1956a and b) and Nikiforuk (1956) have analyzed the soluble and insoluble proteins present; Engel (1948) and Nikiforuk (1956) have detected carbohydrates in the organic material of the enamel.

Like Stack (1955), we felt that the enamel matrix may be composed of more than one substance; and our main attempt has been to separate a series of fractions. It is hoped that purification and histochemical analyses of the various fractions will throw some light on the nature and function of the components of enamel matrix.

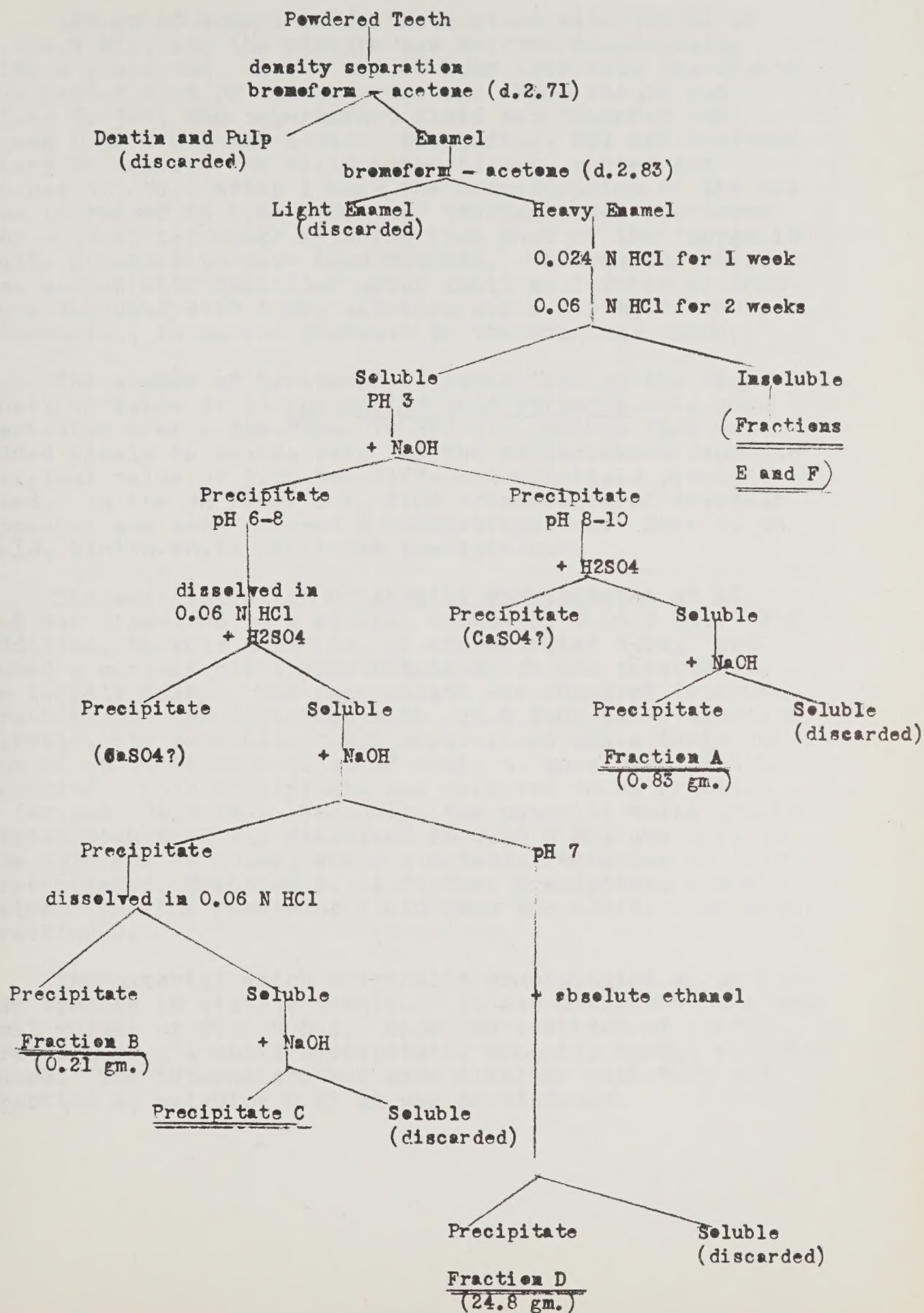
Experimental

Whole teeth of adult cattle were reduced to a powder using a mechanical grinder. The powder was passed through a series of sieves, and only the material which passed through a 60 mesh screen was employed.

The particles of pulp, dentin and enamel were separated from each other by virtue of differences in density (Hodge and Manly, 1939). Pure bromoform (density 2.89 gm/ml) was mixed with small volumes of acetone until a density of 2.71 gm/ml was obtained (checked by weighing on five ml. aliquots on an analytical balance in weighing bottles).

Portions of powdered whole teeth were shaken with 200 ml of the bromoform-acetone mixture and then centrifuged. The residue, which was composed mainly of greyish-white particles overlaid by a small amount of slimy greyish coloured material, was further purified by being centrifuged in a bromoform-acetone mixture of density 2.83 gm/ml. The slimy material remained in suspension and was discarded. The product which was considered to be pure "enamel" was washed with acetone to remove all traces of bromoform, and air-dried. The weight of the enamel powder thus obtained was 155.2 gm.

Table 1. Flow sheet.



125 gm of enamel powder were mixed with 500 ml of 0.024 N HCl, and the mixture was stirred continuously with a glass rod. At 24 and 48 hour intervals the liquid was tested with pH indicator paper. When the pH had risen to 3-5, the supernatant fluid was decanted and fresh 0.02 N HCl was added. Thereafter, HCl was replaced every 24 hours. The fluid extracts were pooled and stored at 4°C. After 1 week the concentration of the HCl was increased to 0.06 N HCl and treatment was continued for another two weeks at which time most of the inorganic salts appeared to have been removed. The residue which was washed with distilled water until no further Cl ions were detected with AgNO₃ solution was a light, fibrous suspension, in marked contrast to the original powder.

The scheme of treatment is summarized by the flow-sheet of Table 1: 1) the pooled acid extracts were concentrated over a low flame to 250 ml. Dilute NaOH was added slowly to neutralize. As the pH increased from the original value of 3.0, two different materials precipitated. In the pH range 6-8, fine cream-coloured crystals appeared and were removed by centrifugation. Then at pH 8-10, bluish-white pellicles precipitated.

The material which originally precipitated at pH 6-8 was dissolved in a minimal volume of 0.06 N HCl. The addition, to this solution, of concentrated H₂SO₄ produced a copious white precipitate which was presumed to be largely CaSO₄. The supernatant was resolved into two fractions by precipitation with .25 N NaOH added dropwise. Firstly, the supernatant was neutralized and a large volume of absolute ethanol added until no more precipitate occurred. This precipitate was referred to as Fraction D (weight, 24.8 gm). Secondly, the material which precipitated with NaOH was dissolved in 0.06 N HCl and left in the cold for 48 hours, where a material weighing 0.21 gm precipitated, Fraction B. A further precipitate was obtained from the remaining fluid upon the addition of NaOH, Fraction C.

The material which originally precipitated at pH 8-10 was treated in similar fashion. It was dissolved in a minimal volume of 0.06 N HCl. Upon the addition of concentrated H₂SO₄, a white precipitate, probably CaSO₄, was produced. The supernatant was made alkaline with NaOH and Fraction A, weighing 0.83 gm was precipitated.

Only Fractions A and D have been analyzed in some detail thus far. Portions of each were dissolved in 0.06 N HCl and dialyzed against cold distilled water overnight. The non-dialysable material was analyzed. The following tests were performed; total sugar by the method of Glegg (1956), hexosamine by the method of Blix (1948), uronic acid by the method of Dische (1947), total N by a Kjeldahl digestion and Nesslerization by the method of Koch and McMeekin (1924), and hydroxyproline by the method of Neuman and Logan (1950). Table 2. In view of the extremely low content of each of the measured substances, and of nitrogen in particular, it was evident that the greater portion of both of the dialyzed fractions was inorganic in nature. In order to evaluate the content of organic material, duplicate samples of both undialysed fractions were ashed to constant weight at 750°C. The loss in weight was assumed to be a measure of the organic moiety. The organic material in "A" was 6.4 and in "D" 4.9 percent by weight.

Table 2

Fraction	Sugar	Hexos- amine	Uronic acid	Nitrogen	Hydroxypro- line
A	1.77	neg.	neg.	0.50	0.015
D	0.21	neg.	neg.	0.02	0.002

(Results expressed as per cent (W/W) of the non-dialysable fraction)

The results in the above table can be reassessed; the nitrogen in "A" is approximately 8 percent of the weight of the organic material; in "D" the nitrogen comprised about 0.4 percent. The low content of nitrogen in "D" is assumed to be due to loss of organic material during dialysis.

2) The fibrous material which remained after treatment of enamel powder with HCl has not yet been analyzed chemically due to difficulties in making it soluble. Under the microscope, it was apparent that this material included two components, an amorphous, brown component and the fibers themselves.

The fibers and the brown amorphous material were tested for solubility in a variety of solvents. Neither was soluble in hot or cold water, hot or cold absolute ethanol, nor cold alcohol-ether. When NaOH solutions ranging in concentration from 0.05 to 10 N were tested at 100°C, it was found that the 10 N NaOH solubilized the amorphous material. As a result the fibers, although split into narrower threads or filaments, were obtained in a purified state (Fraction F). Conversely, concentrated sulfuric acid at room temperature dissolved the fibers, leaving the brown material relatively intact. This material was referred to as Fraction E.

Fractions E and F have not been chemically analyzed. It may be pointed out that the brown substance (Fraction E) reacted strongly with the McManus, Periodic Acid-Schiff (PA-S) stain and the Gomori silver stain and, therefore, seems to be rich in carbohydrates. The fibers (Fraction F) were unresponsive to either technique.

Results and Conclusions

As a working hypothesis, the fibers (Fraction F) are tentatively believed to constitute the core of the enamel prisms. Their extreme resistance to hot concentrated NaOH suggests that they are composed of a highly resistant keratin or perhaps they contain a quite different substance. The splitting of the fibers which was observed in hot concentrated NaOH suggests that an interfibrillar cement is a normal constituent of the fibers.

Whether the brown amorphous material (Fraction E) which is rich in carbohydrates plays a role as an interprismatic cement or whether other fractions are involved in this function can only be speculated at this time.

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APPENDIX J

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: The organic components of dental tissues.

Grantee: Dr. G. Nikiforuk (with R. C. Burgess),
Div. of Dental Research, University of
Toronto.

Project No.: D.R. 57 Amount of Grant: \$2,980.00

SUMMARY OF WORK

1. For qualitative amino acid determinations a solvent of 2-butanol:2 per cent ammonia (150:60 V/V) in one dimension and 2-butanol:formic acid:water (150:30:20 V/V) in the second dimension was found most suitable.
2. The R_f values for standard sugars in various aliphatic and aromatic solvents are described in Table 1. 2-butanol and 2 per cent ammonia (150:60 V/V) is a satisfactory solvent for separation of sugars generally found in biological tissues.
3. Hydrolysis of standard sugars in Dowex 50-X12 at 80°C for 96 hours did not result in any significant destruction of glucuronic acid, glucose, mannose and galactose (Fig. 5). Fucose, a highly labile aldohexose, was recovered in appreciable quantities.
4. A water soluble ninhydrin-positive material may be extracted from powdered enamel.
5. The dominant hexoses in enamel in decreasing order of concentration are galactose, glucose, and mannose.
6. Uronic acid does not appear to be a major component of the polysaccharide of enamel.
7. The qualitative pattern of the carbohydrates of enamel appears to be akin to those tissues which are rich in reticular fibers.

PROGRESS REPORT

This report deals with further investigations of the carbohydrate and amino acid composition of enamel using paper chromatography techniques. During the past year considerable emphasis was placed on improvement of chromatographic techniques.

Methods and Material

Only a summary of the methods used is included in the present report since the last report to the Committee, March, 1956, described in detail the techniques used. Where new procedures were involved a more detailed account follows.

1. Preparation and Purification of Enamel

Cleaned teeth were pulverized in a mill to pass a 100 mesh screen. A flotation technique using a bromoform-acetone mixture having a density of 2.75 is used to separate enamel (S.G. 2.90) from dentin (S.G. 2.14). After four to eight separations powder containing 99.9% enamel was obtained. Purity was assessed by counting the enamel and dentin particles. Enamel particles were differentiated from dentin on the basis of different refractive indices (Manly and Hodge, 1939).

2. Demineralization

The enamel powder was demineralized in a cellophane membrane against ethylene diamine tetraacetate (pH 7.1) and separated into a soluble and insoluble fraction by centrifugation.

3. Hydrolysis

The insoluble fraction was hydrolysed in a sealed ampule using 5 ml. of 6N HCl for 48 hours at 110°C for amino acid determinations. For carbohydrate determinations hydrolysis was carried out with a cation exchange resin (Dowex 50 - X12) at 80°C for 96 hours. The soluble carbohydrate was separated from the resin by centrifugation and washing. Hexosamines were absorbed on the resin and were eluted with 2.0N HCl.

4. Chromatography Technique.

(a) Amino Acids.

The solvents used for amino acid resolution were: (1) n-isopropyl alcohol and water (80:20, V/V), and (2) phenol (saturated with an aqueous solution containing 6.3% sodium dihydrogen phosphate) containing 5 per cent ethyl acetate (V/V). The main disadvantage of this system is that leucine and isoleucine, valine and methionine are not separated.

Attempts were made to employ other solvents in the two-dimensional development of the amino acid papergrams. Figures 1 to 3 show developed papergrams using the following solvents, respectively:

- (1) 1. Propanol:water:propionic acid (20:5:2, v/v).
2. N-butanol:acetone:water:dicyclohexylamine (10:10:5:2, v/v) (Hardy, Holland and Nayler, 1955).
- (2) 1. Propanol:water:propionic acid (20:5:2, v/v).
2. 2-butanol:2 per cent ammonia (150:60, v/v).
- (3) 1. 2-butanol:2 per cent ammonia (150:60, v/v).
2. 2-butanol:formic acid:water (150:30:20, v/v). (modified from Hausmann, 1952).

The best separation of amino acids was obtained by using the last named solvents (Fig. 3). It was found that a double development in the 2-butanol:2 per cent ammonia in the first dimension resulted in smaller spots and better separation. This solvent system, while very satisfactory for qualitative work, was found to be unsuitable for quantitative determination. The presence of ammonia interfered significantly with the ninhydrin reaction. An attempt was made to remove the ammonia by spraying the paper with a 1 per cent solution of KOH in absolute methanol according to the method of Parnas and Wunderley, 1953. This procedure proved inadequate for our purpose. Flooding the paper spots with borate buffer, pH 11.5, did not remove the ammonia sufficiently to proceed with quantitative analysis. It was considered that the ammonia, fixed by the carboxyl groups of the paper and by the solvent (as in formic acid) could not be sufficiently removed by the use of alkali buffers.

The use of these solvents was therefore discontinued for quantitative analysis of amino acids. Further studies are at present underway to overcome these difficulties.

(b) Sugars.

In the previous report, March 1956, the solvents used for developing sugar chromatograms were N-butanol:pyridine:water (3:1:1, V/V). Apart from the pungent odour of pyridine, another disadvantage of this solvent is the low R_f values for the uronic acids. These substances remain close to the origin, and reading of the papergram may present difficulties. Accordingly, other solvents were tested for separation of the major carbohydrates that have been reported in biological tissues. Table 1 summarizes the R_f values for the various carbohydrates in different solvents. It has been observed that the actual order of separation is very much the same in most aliphatic solvents (Isherwood, 1954), except for solvents containing phenol. Table 1 shows that solvents containing other aromatic solvents, i.e. morpholine, aniline, resorcinol and cyclohexylamine, gave a different pattern of separation and can be used to provide an additional chromatographic criterion as to identity of an unknown sugar. There were some undesirable effects, such as spot elongation. The results indicate, however, that aromatic solvents may have promise in sugar chromatography.

A solvent containing 2-butanol:2 per cent ammonia (150:60) satisfactorily separated glucuronic acid, galactose, glucose, mannose, and fucose (Fig. 4). A distinct advantage of this solvent over the pyridine:butanol:water solvents is that glucosamine ($R_f = 0.507$) and galactosamine ($R_f = 0.450$) are clearly separated. Valuable features of the solvent containing N-propanol:propionic acid:water are that galacturonic acid moves well away from the origin ($R_f = 0.18$) and that glucuronic acid runs in the lactose form only ($R_f = 0.515$). As a general rule it was observed that a double development improved the separation of the sugars and resulted in more compact spots, as noted in Table 1.

Findings and Discussion.

1. Hydrolysis experiments.

Hydrolysis conditions for substances containing carbohydrates are very critical in that considerable destruction of these substances occurs. Standard preparations containing fucose, glucose and mannose,

glucuronic acid, and glucosamine were hydrolyzed using an ion exchange resin, Dowex 50 - X12, for 24, 48, 72, and 98 hours at 80°C. Papergrams of these solutions developed in pyridine:butanol, when compared with standards, indicated that no apparent destruction of the sugars had occurred (Fig. 5). These results are most encouraging in that quantitative analysis for sugar components of various tissues after hydrolysis may be relied upon to a greater extent than in most previous work in this field.

Experiments were set up to determine whether the above hydrolysis conditions result in interconversion of one sugar to another. It was found that galactosamine and glucosamine were recovered after eluting the resin with 2.0N HCl. The N-acetyl glucosamine is entirely converted into glucosamine. No interconversion of the hexoses or the uronic acids occurred.

2. Soluble Organic Fraction.

Enamel powder in a cellophane membrane after demineralization and washing is divided in a soluble and insoluble fraction. The report of March 1956, noted that ninhydrin positive contaminants from the cellophane membrane prevented positive identification of a soluble protein or peptide fraction. Further results are now reported on the soluble fraction.

Quantitative analysis of cellophane contents from an experimental and control suggest that soluble ninhydrin positive material is extracted from the enamel. When 1.506 grams of enamel are decalcified as above, the ninhydrin soluble components in the control bag are equivalent to 0.188 μ M of leucine and the experimental contained 3.960 μ M of leucine equivalents. When 0.995 grams of enamel powder are placed in a container in a shaker for three days at room temperature, the amount of ninhydrin positive material extracted is equivalent to 0.164 μ M of leucine. When the same enamel is further diluted in 30 cc. of water and placed in a boiling water bath, for 20 minutes, a further ninhydrin positive fraction is extracted, which is equivalent to 3.94 μ M of leucine.

The soluble fraction in view of its non-diffusible character is probably of a peptide nature. A communication from Irving (1956) suggests that the soluble components may be associated with the milling of the enamel

into powder. This explanation has not been subjected to experiment in our laboratory.

3. Carbohydrate Components in Enamel (Soluble and Insoluble Fractions).

Further analyses of the carbohydrate moieties of the insoluble enamel fraction have been carried out. Fig. 6A and 6B is a chromatogram in n-butanol:2 per cent ammonia and in n-butanol:pyridine:water solvent of the hexoses of this fraction. The dominant hexoses in enamel in decreasing order of concentration are galactose, glucose and mannose. No definite chromatographic evidence for fucose was found. Its present cannot yet be excluded since the quantities of enamel in each experiment were not over five grams. Trace quantities of a uronic acid (probably glucuronic) may be present. The qualitative pattern for the soluble fraction of enamel appears similar to the insoluble (Fig. 6A and B). There are, however, no free carbohydrate components in either fraction since unhydrolyzed soluble and insoluble enamel fractions did not yield any reducing sugars on the papergram.

Trace quantities of uronic acid may be present but it does not appear to be a principal component of the polysaccharides in enamel. It is interesting to note that so far the cornea is the only tissue in the animal kingdom known to contain an amino-polysaccharide which does not contain uronic acid as a principal component (Meyer et al, 1953). The name keratosulphate has been proposed for this distinct amino-polysaccharide. Further studies on larger amounts of enamel (up to five grams have been used in this study) should provide positive evidence as to presence or absence of this component.

The hexosamine fraction was eluted from the resin with 2.0N HCl and chromatographed. Considerable difficulty was encountered in the chromatography and colour reaction for the hexosamine, due to the fact that the amino acids were also eluted by this procedure. Where considerable quantities of the amino acid were present interference in the colour reaction when aniline was used was noted, hence masking any hexosamine that was present. When the papergrams were tested for an amino sugar according to the method of Morgan and Elson (1933), as noted by Partidge (1947), no conclusive chromatographic evidence for the presence of a hexosamine in the enamel was observed. Further work is required in this connection.

It appears that the qualitative pattern of the carbohydrate components of enamel is most akin to those tissues which are rich in reticular fibers, i.e. lung, lymph node, testes and tendon (Glegg et al, 1953). No free hexoses could be detected on the chromatogram of unhydrolyzed soluble or insoluble enamel fractions. These components most probably exist in association with a protein.

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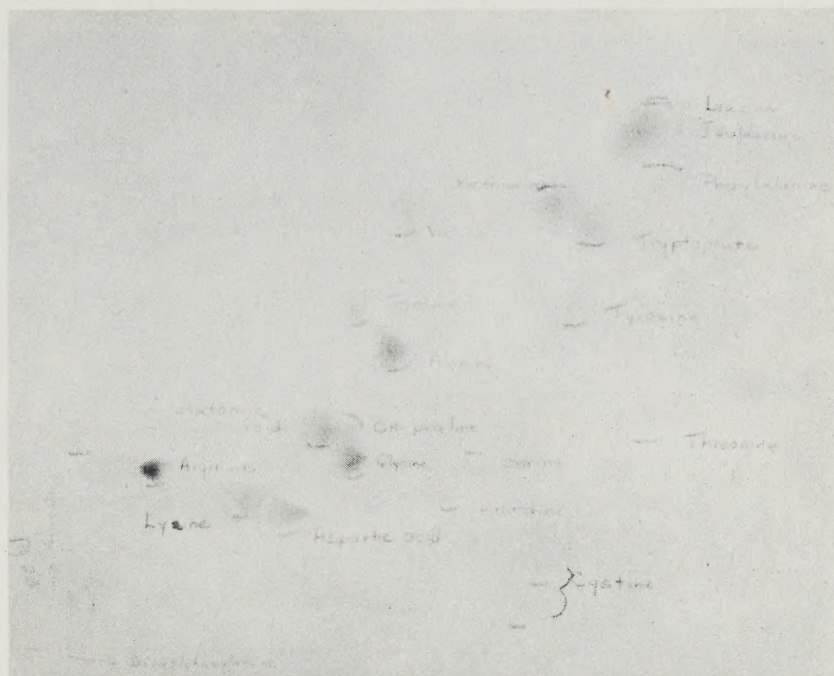


FIG. 1. A 2-DIMENSIONAL PAPERGRAM OF STANDARD AMINO ACIDS
 USING THE FOLLOWING SOLVENTS:
 (1) N-PROPANOL:PROPIONIC ACID:WATER (72:8:20 v/v)
 (2) N-BUTANOL:ACETONE:WATER:DICYCLOHEXYLAMINE
 (10:10:5:2 v/v).

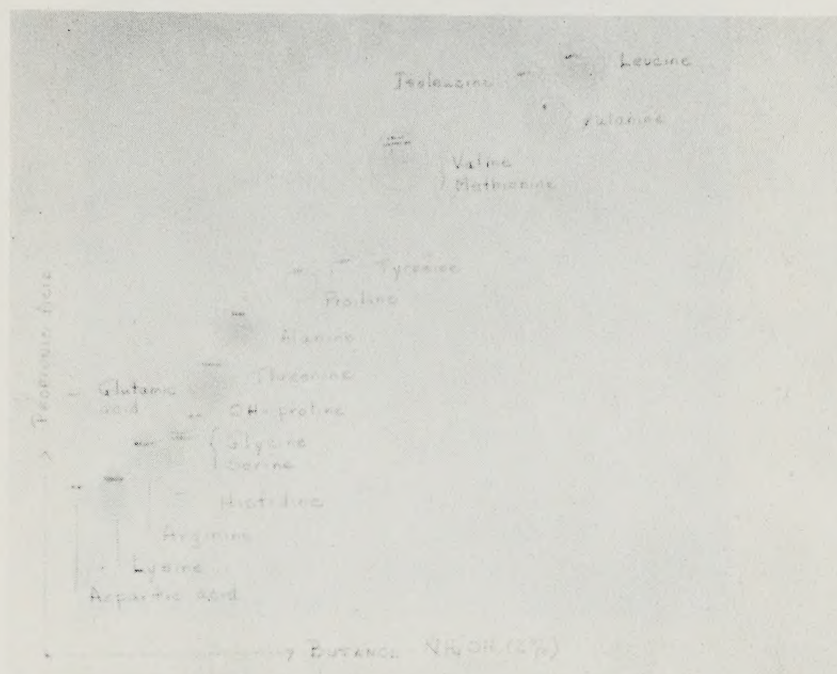


FIG. 2. A 2-DIMENSIONAL PAPERGRAM OF STANDARD AMINO ACIDS USING THE FOLLOWING SOLVENTS:
 (1) 2-BUTANOL:2 PER CENT AMMONIA (150:60 v/v)
 (2) N-PROPANOL:PROPIONIC ACID:WATER (72:8:20 v/v)

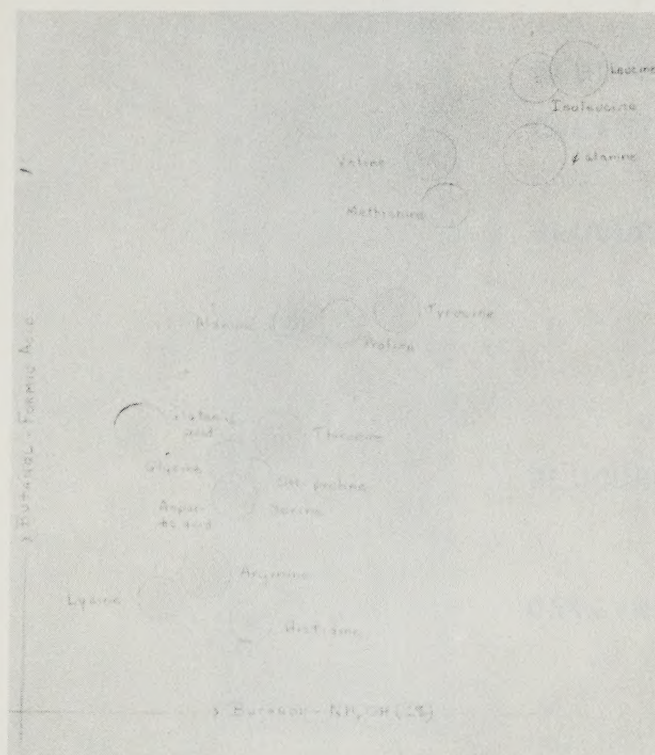
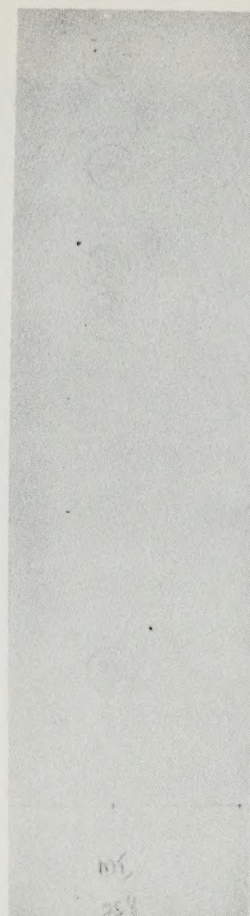


FIG. 3. A 2-DIMENSIONAL PAPERGRAM OF STANDARD AMINO ACIDS USING THE FOLLOWING SOLVENTS:
 (1) 2-BUTANOL:2 PER CENT AMMONIA (150:60 v/v)
 (2) 2-BUTANOL:FORMIC ACID:WATER (150:30:20 v/v).

J



FUCOSE

MANNOSE

GLUCOSE

GALACTOSE

GLUCURONO LACTONE

GLUCURONIC ACID

ORIGIN

FIGURE 4. SEPARATION OF STANDARD SUGARS IN A 2-BUTANOL: 2 PER CENT AMMONIA (150:60 V/V) SOLVENT AFTER TWO DEVELOPMENTS.

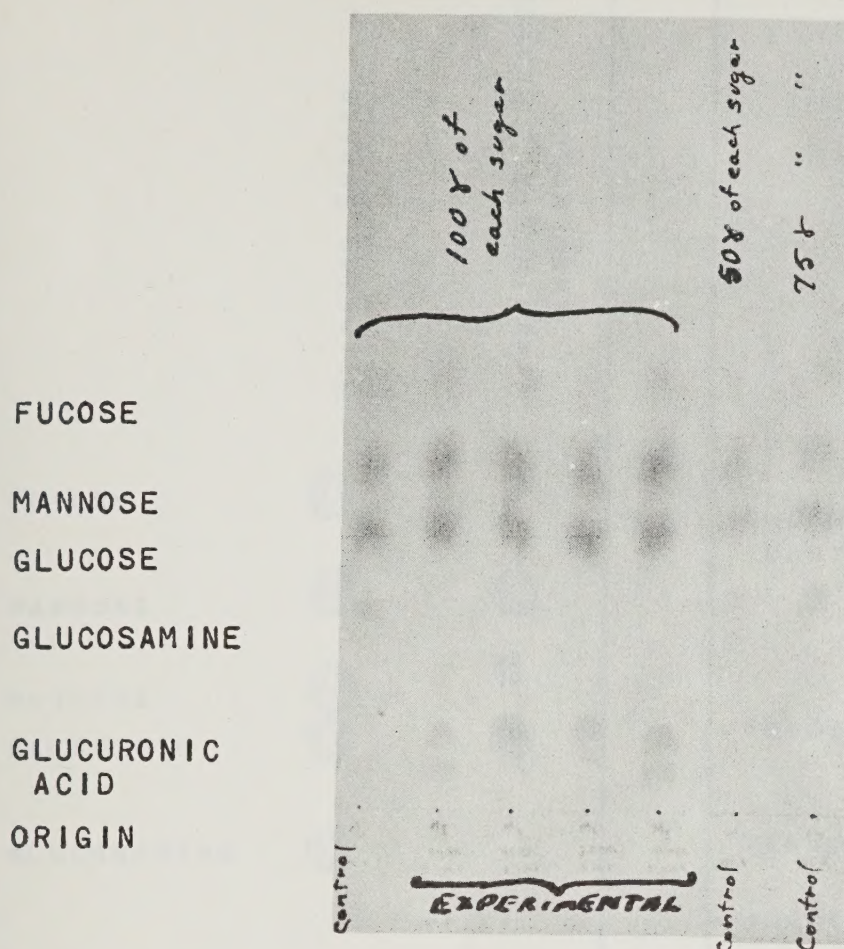


FIGURE 5. PAPERGRAM OF THE FOLLOWING SUGARS: GLUCURONIC ACID, GLUCOSAMINE, GLUCOSE, MANNOSE, AND FUCOSE, BEFORE AND AFTER HYDROLYSIS IN DOWEX 50-X12 AT 80°C FOR 24, 48, 72 AND 96 HOURS. NOTE THAT THE DENSITY OF THE SPOT IS UNAFFECTED BY HYDROLYSIS OF THE HEXOSES. THE GLUCOSAMINE REMAINS ON THE RESIN AFTER HYDROLYSIS AND IS THEREFORE NOT PRESENT IN THE EXPERIMENTAL COLUMNS OF THE PAPERGRAM.

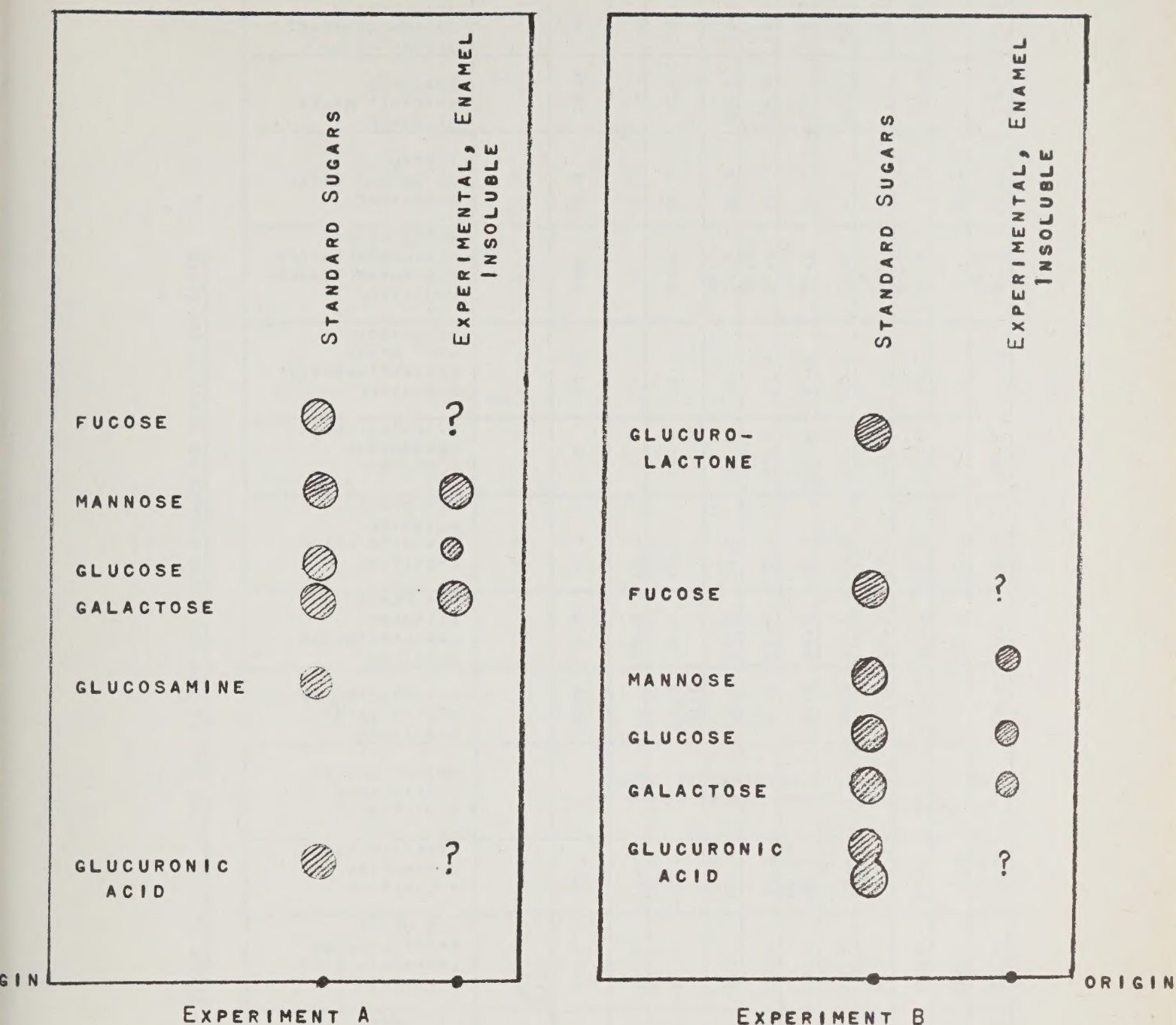


FIG. 6A AND B. COMPOSITE TRACING FROM ORIGINAL PAPERGRAMS TO INDICATE CARBOHYDRATE COMPONENTS IN THE INSOLUBLE ORGANIC FRACTIONS OF ENAMEL. IN EXPERIMENT A, THE PAPERGRAM WAS DEVELOPED IN BUTANOL-AMMONIA (2%); IN EXPERIMENT B, THE PAPERGRAM WAS DEVELOPED IN PYRIDINE:BUTANOL:WATER SOLVENT. PAPERGRAMS FROM THE SOLUBLE FRACTION WERE QUALITATIVELY SIMILAR.

TABLE I. Rf x 100 VALUES FOR STANDARD SUGARS IN VARIOUS SOLVENTS

	N-PROPANOL WATER 80:20	N-PROPANOL ACETIC ACID WATER, 70:10:20	N-PROPANOL PROPIONIC ACID WATER, 72:8:20 (1 DEV.)	N-PROPANOL PROPIONIC ACID WATER, 72:8:20 (2 DEV.)	N-PROPANOL MORPHOLINE WATER, 72:8:20	3-BUTANOL, FORMIC ACID WATER, 75:1:24	N-BUTANOL ACETIC ACID, WATER, 60:20:20	N-BUTANOL PYRIDINE, WATER 60:20:20 (1 DEV.)	N-BUTANOL PYRIDINE, WATER 60:20:20 (2 DEV.)	N-BUTANOL RESORCINOL WATER, 60:20:20	N-BUTANOL ETHANOL, RESORCINOL, WATER, 30:30:20:20	N-BUTANOL N-PROPANOL, CYCLO- HEXYLAMINE, WATER 35:35:10:20	2-BUTANOL 2% NH ₄ OH, WATER 150:60	METHANOL ANILINE, WATER 25:55:20	PHENOL, 6.3% SODIUM CITRATE, 3.7% SODIUM DIHYDROGEN PHOS- PHATE
GALACTURONIC ACID	-	-	18.0	27.7 - 31.3	38.0		-	-	4.0	-	-	-	16.2	-	-
GLUCURONIC ACID	NONE	NONE	NONE	NONE	39.6		NONE	2.75	4.4	3.0	11.3	NONE	18.9	NONE	6.7
GALACTOSAMINE	-	-	24.2	38.8	QUERY		-	12.5	16.0	-	-	-	45.0	-	-
GLUCOSAMINE	33.5	37.6	27.2 -32.3	43.5 -50.6	59.0 -64.4		24.8 - 30.4	15.5	19.7	19.3	33.0	34.4	50.7	4.4	39.1
GALACTOSE	35.0	40.4	30.9	47.1	65.0 - 69.0		29.2	23.7	31.7	24.7	38.3	64.7 - 75.6	42.9	69.3	49.0
GLUCOSE	38.2	43.4	32.8	50.6	58.2		30.2	28.6	37.5	25.5	39.4	59.6 - 67.2	45.9	69.5	44.0
MANNOSE	43.0	48.6	38.6	57.3	66.0 - 69.0		35.3	34.5	45.5	31.4	43.2	62.4 - 72.2	53.7	71.4	50.7
FRUCTOSE	43.4	48.4	39.0	58.8	60.4		36.5	35.6	45.5	35.1	46.0	38.2	51.0	33.8	59.0
N-ACETYL GLUCOSAMINE	50.7	55.7	-	-	-		40.3	40.3	51.1	46.9	-	-	-	-	75.2
FUCOSE	50.4	56.8	50.1	69.5	79.6		45.8	47.1	58.2	46.5	53.7	74.8 - 81.9	59.1	79.8	66.2
RIBOSE	-	-	49.7	69.5	76.8		-	50.4	62.2	-	-	-	62.4	-	-
GLUCURONIC LACTONE	55.1	60.6	51.5	73.3	66.0 - 71.0		48.3	64.0	75.9	46.9	52.8	48.5 - 58.4	34.5	73.7	64.3

APPENDIX K

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: Investigations into the nature of cementum.

Grantee: Dr. K.J. Paynter,
Division of Dental Research,
University of Toronto.

Project No.: D.R. 64 Amount of Grant: \$3,000.00

SUMMARY OF WORK

Cementum on the molar teeth of rats has been studied using a variety of histological and histochemical methods. Rats were sacrificed at ages between 20 and 100 days, their jaws fixed in 10% formalin and successive longitudinal sections through the teeth and mandible were treated with H. & E., a collagen stain, a silver stain, P.A.S., Alcian blue, Millon's reaction, Toluidine blue, and thionine following ribonuclease. Studies indicate that acellular cementum is composed of two layers, an inner amorphous ground substance 1.5 - 2.0 microns thick, with staining reactions similar to the ground substance of cartilage. This material forms originally as a basement membrane between the pulpal surface of the epithelial diaphragm of the developing root and the pulp itself. The second and outer layer of acellular cementum is deposited on the surface of the first, is composed of a similar ground substance, and contains the embedded ends of the periodontal membrane fibres. Cytoplasmic R.N.A. in cementoblasts forming cellular cementum has been demonstrated.

The outer layer of cementum is attached to the amorphous substance just described; its thickness varies, becoming thinner with age. It is the substance which forms the actual attachment of the periodontal fibers to the tooth. The staining reaction of the ground substance of this material is worked by the heavy bundles of collagen within it. These bundles penetrate its full thickness, but do not go beyond this, i.e. they do not enter the amorphous layer just described.

The fibrous outer layer stains positively for collagen.

PROGRESS REPORT

Cementum is a calcified connective tissue covering the anatomical roots of the teeth of man and many animals. It is present in two forms, cellular and acellular, depending on the presence or absence of cells within the matrix of the material. Acellular cementum is generally confined to the coronal one-third to one-half of the root; the rest is covered with cellular cementum.

This investigation has been largely directed toward finding out more about the nature and the formation of acellular cementum in the rat, using histological and histochemical methods. Albino rats of the Carworth strain were sacrificed at approximately 15, 20, 50, 60, 75 and 100 days of age. Jaws were dissected free, fixed in 10% formalin, and subsequently decalcified using formic acid, and sodium citrate. Sections were made in paraffin at six microns, and adjacent sections from each animal treated with hematoxylin and eosin, Romeis' collagen stain, silver stain, P.A.S. reaction, Alcian blue, Millon's reaction, toluidine blue, thionine, and thionine following treatment with ribonuclease.

Observations

(a) Structure of Acellular Cementum.

This material is composed of two distinct layers. The thinner layer next to the dentin of the root is about 1.5 to 2 microns thick, and is non-fibrous as far as can be ascertained (Figs. 3A and 3B). It stains deep blue with hematoxylin, is positive to P.A.S. (Figs. 2A and 2B) and to Alcian blue. It is highly metachromatic (Fig. 1B) simulating the ground substance of cartilage in this regard. It does not stain positively for either collagen or reticular fiber (Fig. 3B).

The outer layer of cementum is attached to the amorphous substance just described; its thickness varies, becoming thicker with age. It is the substance which forms the actual attachment of the periodontal fibers to the tooth. The staining reaction of the ground substance of the material is masked by the heavy bundles of collagen within it. These bundles penetrate its full thickness, but do not go beyond this, i.e. they do not enter the amorphous layer just described.

The fibrous outer layer stains positively for collagen.

The ground substance is P.A.S. positive, and metachromatic, but was not found to be positive to Alcian blue, although this is probably because a red collagen stain was used as a counter stain, and this may have masked the reaction.

(b) Development of Acellular Cementum.

During the development of teeth, after the shape of the crown has been outlined by the cells of the enamel organ, and after a considerable amount of hard tissue has been deposited in this area, the cells forming the cervical loop, i.e. the lower border of the enamel organ at the junction of the cells of the inner and outer enamel epithelia, begin to grow down through the underlying mesenchyme. These ectodermal-derived cells are responsible for organizing cells of the dental papilla so that they differentiate into odontoblasts in the root portion of the tooth. The proliferating epithelial cells form a thin band around the outside of the root, and form an incompletely closed diaphragm over the apical end of the developing root. The band and diaphragm comprise Hertwig's epithelial root sheath. The band of cells does not remain complete along the whole length of the root. Instead it breaks up after development of the root has progressed to a certain point. Thus the root sheath can only be observed as a complete entity in the region of the developing end of the root.

Along the surface of the epithelial diaphragm facing the future pulp of the tooth, a thin basement membrane is discernable. The material of this basement membrane is positive to P.A.S. and to Alcian blue, basophilic, but not metachromatic (Figs. 1C and 2C, see arrows). No reticular fibers could be distinguished in it. However, on the lower, opposite side of the root diaphragm, i.e. the side facing away from the future pulp, a dense network of argyrophilic fibers could be distinguished (Fig. 3C).

The basement membrane continues across the top of the diaphragm and up along the outer surface of the root. It becomes the amorphous inner layer of the acellular dentin described above. A short distance above the developing tip of the root it becomes metachromatic. This change is probably related to calcification, although this has not been confirmed.

The outer or fibrous layer of acellular cementum appears on the tooth somewhat later. It was not recognized at 20 days on the rat first molar, but could easily be seen

at 50 days. Special cells responsible for its deposition could not be distinguished, although this may have been because they were masked by the very thick bundles of collagen in the area. Further work is being attempted to clarify the presence or absence of such cells.

(c) Cellular Cementum.

This material does not appear on the root surface until about the time of tooth eruption. It forms quickly and in considerable amounts. The material is characterized by the presence of heavy collagen bundles penetrating through the ground substance. The latter is histochemically similar to that of bone. The cells which form cellular cementum - cementoblasts - are very similar to osteoblasts in appearance. They were observed in large numbers in areas where the material was being deposited. They can be recognized by their large open-faced nuclei, prominent nucleoli, and dark basophilic cytoplasm. A considerable part of the cytoplasmic basophilia was due to the presence of ribonucleic acid, as indicated by its disappearance following treatment of sections with ribonuclease.

(d) Other Studies.

Attempts to measure the thickness of cellular cementum in rats have not led to definite conclusions. This was attempted, it may be recalled from the original application, in order to ascertain whether there was anything unique about the canalicular mechanism within the material. This in turn related to the vitality of the cells within the lacunae. Further efforts along this line are anticipated in future studies.

Experiments involving implantation of various tooth tissues into the subcutaneous tissues of the rat also have been quite unproductive. Nothing of significance can be reported at this time.

(d) Discussion.

The inner amorphous layer of acellular cementum described above is of particular interest. Histochemically it reacts both in selectivity and intensity, in a manner similar to the ground substance of cartilage. It would appear to be a sulfated acid mucopolysaccharide apparently similar to chondroitin sulfuric acid. It is continuous with the basement membrane covering the dentin of the

crown under the enamel. This latter membrane appears on the tooth between ameloblasts and odontoblasts just prior to hard tissue formation. Similarly, the layer of cementum first is apparent as a basement membrane between cells of the root sheath and cells of the dental pulp. The cells of the root sheath concerned in its production are from the layer of the enamel organ that gives rise to ameloblasts, i.e. the inner enamel epithelium.

It appears further that this material forms the tissue of attachment for the oral epithelium and the tooth at the cervix, and that the 'epithelial attachment' is similar to the attachment of epithelium to connective tissue elsewhere in the body.

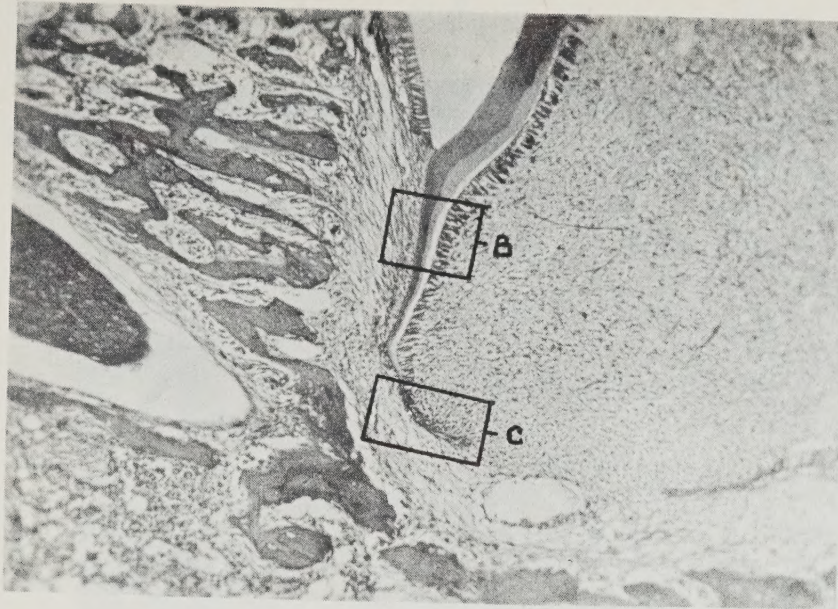
(f) Future Investigation.

Future studies into the nature of cementum will involve three investigations to be carried on simultaneously. First, using human teeth, further measurements will be made of the thickness of cellular cementum, and at the same time an attempt will be made to establish the state of vitality of cells within the material. (Doubt has arisen as to whether the deepest cells in the matrix are living or dead.)

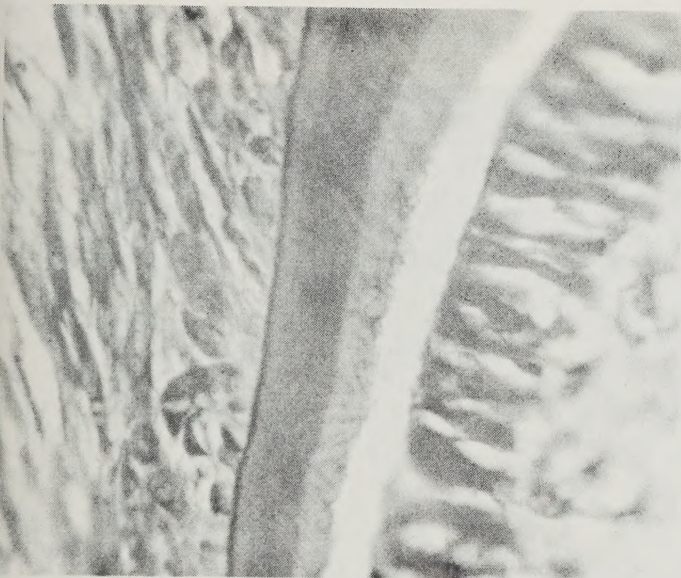
Secondly, more animals will be studied at intermediary ages to those reported here, using the above-mentioned histochemical procedures, in order to fill in the sequence of development more fully. In addition it is proposed, under the direction of Dr. A.F. Howatson of the Department of Anatomy, University of Toronto, to carry out an electron microscope investigation of acellular cementum, to establish particularly the structure of the amorphous layer next to the dentin.

Finally, it is proposed to study the healing process in cementum following injury. In this experiment the fibers of the periodontal membrane will be severed from one side of the root of a molar in the rat. Then by sacrificing animals at various time intervals following injury, the healing process may be followed to its conclusion. This should lead to a better understanding of cells responsible for cementum production.

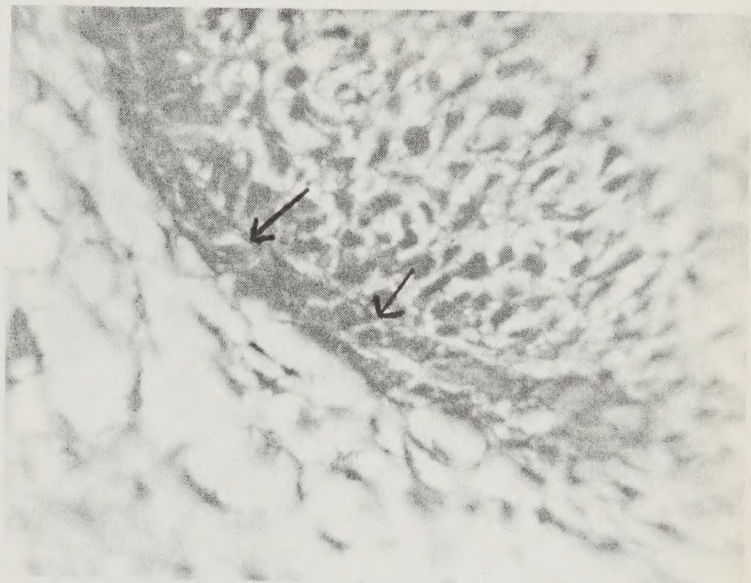
K



A



B



C

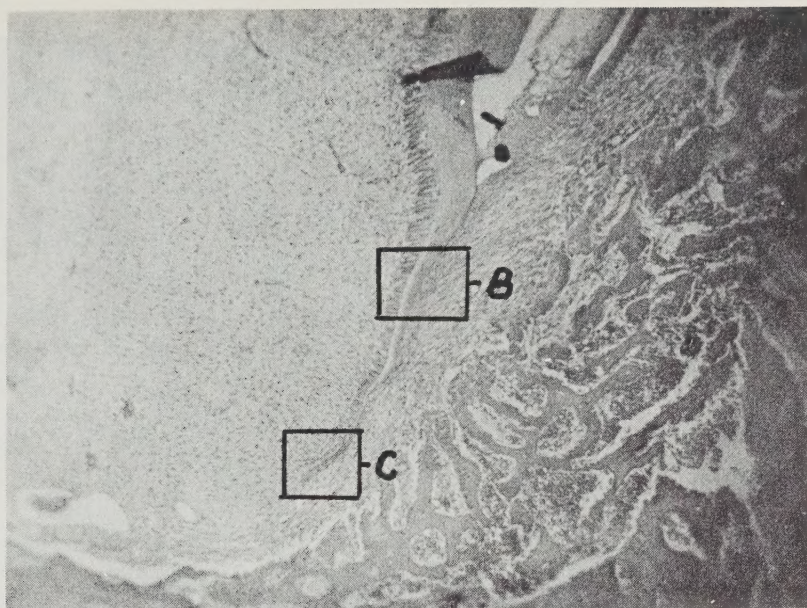
FIG. 1A - LOW POWER PHOTOMICROGRAPH OF THE DEVELOPING ROOT OF A LOWER FIRST MOLAR FROM A RAT 14 DAYS OLD. TOLUIDINE BLUE STAIN.

FIG. 1B - HIGH POWER PHOTOMICROGRAPH FROM THE AREA LABELLED B IN FIG. 1A.

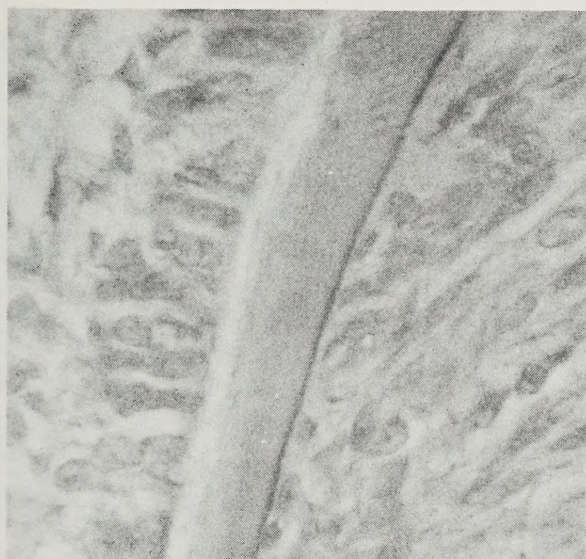
FIG. 1C - HIGH POWER PHOTOMICROGRAPH FROM THE AREA LABELLED C IN FIG. 1A.

(ARROWS POINT TO THE BASEMENT MEMBRANE)

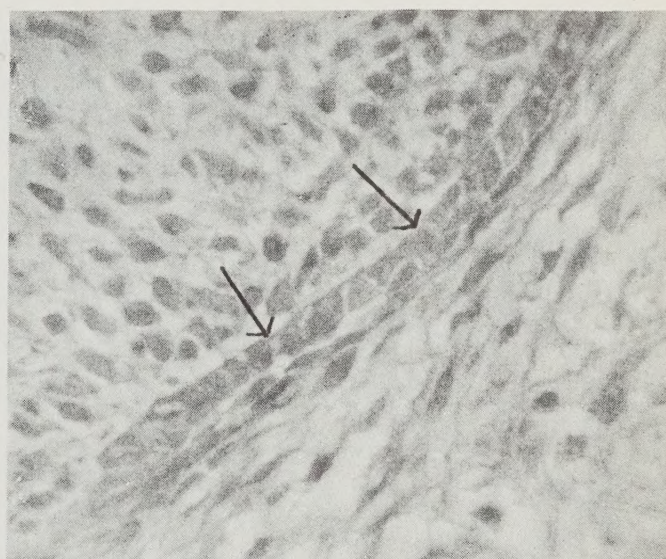
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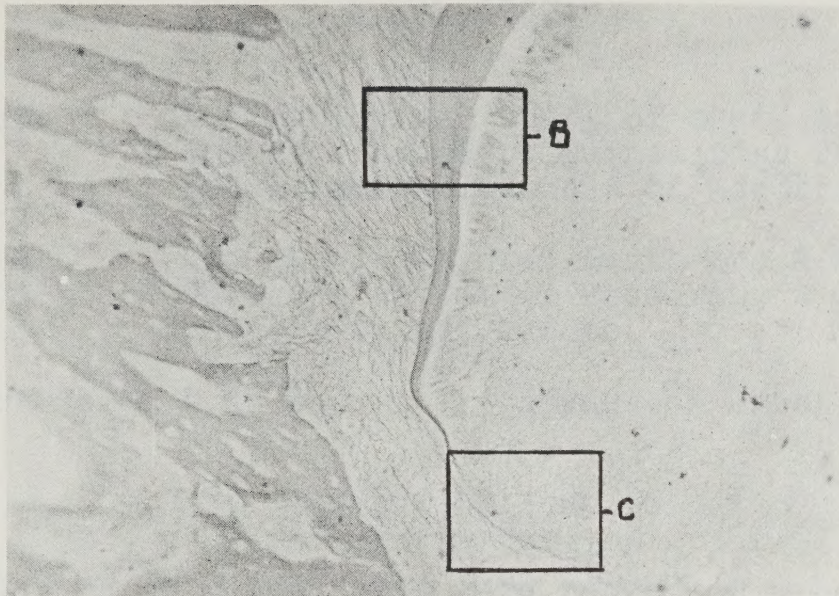
FIG.2A - LOW POWER PHOTOMICROGRAPH OF THE DEVELOPING ROOT OF A LOWER FIRST MOLAR FROM A RAT 14 DAYS OLD. PERIODIC ACID-SCHIFF STAIN.

FIG.2B - HIGH POWER PHOTOMICROGRAPH FROM THE AREA LABELLED B IN FIG.1A.

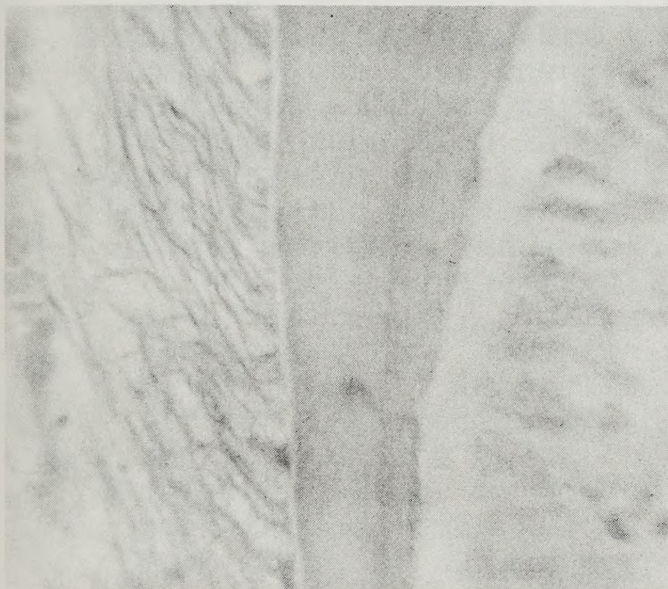
FIG.2C - HIGH POWER PHOTOMICROGRAPH FROM THE AREA LABELLED C IN FIG.1A.

(ARROWS POINT TO THE BASEMENT MEMBRANE)

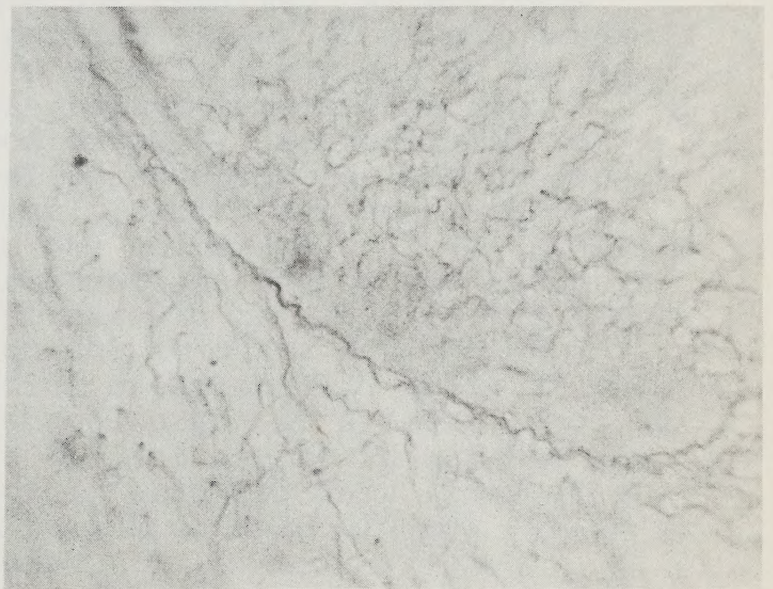
K



A



B



C

- FIG. 3A - LOWER POWER PHOTOMICROGRAPH OF THE DEVELOPING ROOT OF A LOWER FIRST MOLAR FROM A RAT 14 DAYS OLD. SILVER STAIN.
- FIG. 3B - HIGH POWER PHOTOMICROGRAPH FROM THE AREA LABELLED B IN FIG. 3A.
- FIG. 3C - HIGH POWER PHOTOMICROGRAPH FROM THE AREA LABELLED C IN FIG. 3C.

APPENDIX L

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: A histological study of teeth and periodontal tissues of guinea pigs reared on subminimal allowances of ascorbic acid.

Grantee: Dr. K. J. Paynter (with Dr. A.M. Hunt)
Division of Dental Research,
University of Toronto.

Project No.: D.R. 63 Amount of Grant: \$1,455.00

SUMMARY OF WORK

Groups of guinea pigs were reared on a basal synthetic diet. The ascorbic acid content of the diet was varied from 0.0 to 5.0 mg. per animal per day. Appropriate ad lib. and pair fed controls were employed. Histologic sections of teeth and jaws, treated with a variety of stains, were used to evaluate the results. Alterations in growing dentin were the earliest signs of stress observed. Minor bone changes were seen in the group on 0.4 mg. ascorbic acid per day. These became more severe with further reduction in the food element. By carefully recording the normal pattern of bone deposition and resorption in the walls of the alveoli, which accompany normal tooth movement and growth in the guinea pig, it was possible to evaluate more carefully alterations due to the ascorbic acid deficiency. Collagen fibres did not form in the periodontal membrane of animals on the absolute deficiency, which resulted in proliferation of epithelial cells of the stratum intermedium of the enamel organ at a lower level on the side of the tooth. This did not happen in animals receiving 0.4 mg. or more ascorbic acid per day. Tissues of pair fed controls to the deficient animals did not differ from those of ad lib. controls.

PROGRESS REPORT

The purpose of the experiment was to determine the effects of severe and moderately severe deficiencies of ascorbic acid on the tissues of the jaws and molar teeth of guinea pigs when a synthetic diet was used as the basal feeding.

Materials and Method

Guinea pigs, approximately five weeks old, and ranging in weight from 180 to 250 grams, were assigned at random to one of five groups depending on (a) the amount of ascorbic acid administered daily, (b) the period in days of the experimental feeding, and (c) the type of feeding (ad lib. or pair fed). Treatment of the various groups is shown in Table 1.

The synthetic diet of Reid and Briggs (1), minus ascorbic acid, was used as the basal diet. Ascorbic acid was administered daily in the required amounts using accurately equilibrated syringes. Pair feeding methods were used to estimate tissue changes due to inanition alone.

At sacrifice the head of each animal was fixed in Bouin's. Hard tissues were decalcified in equal parts of 45% formic acid and 20% sodium citrate. Histological sections included the following:

- (a) Longitudinal sections of upper and lower right jaws and molar teeth;
- (b) Cross sections of upper and lower left jaws and molar teeth at three levels.

Blocks were embedded in paraffin. Six adjacent sections were cut in each of these areas, and stained with hematoxylin and eosin, toluidine blue, Van Geisen collagen stain, and Conceiro and Friere's silver stain. In addition dried skulls of normal animals of various ages were examine. Daily weight records were kept.

Experimental FindingsA. Weight recordsLegend

Graph 1 shows comparison of daily weights of group IIA, 5 mgs. ascorbic acid daily, ad lib.

IIIA, 5 mgs. ascorbic acid daily,
pair fed to IVA

IVA, 0.4 mgs. ascorbic acid daily,
ad lib.

Graph 2 shows comparison of daily weights group IIB, 5 mgs. ascorbic acid daily, ad lib.

IIIB, 5 mgs. ascorbic acid daily,
pair fed to IVB.

IVB, 0.4 mgs. ascorbic acid daily,
ad lib.

Graph 3 records daily weight gains for group V. Legend: = 0.4 mgs. ascorbic acid daily, ad lib. feeding - 48 days; _____ = 5.0 mgs. ascorbic acid daily, ad lib. feeding - 27 days.

Differences in growth rate, as indicated by weight gains (Graphs 1 and 2) show that animals in groups IVA and IVB receiving 0.4 mgs. ascorbic acid daily gained less than their pair fed mates (Groups IIIA and IIIB), which had eaten the same amount of food but had received 5.0 mgs. ascorbic acid. These in turn had gained less than animals in groups IIA and IIB which had eaten ad lib. and received 5.0 mgs. ascorbic acid daily.

Graph 3 shows increase in daily weight gains of animals in group V once they had been restored to 5.0 mgs. ascorbic acid daily.

B. Enamel Organ Cells: Ameloblasts and stratum intermedium cells).

Guinea pigs have continually erupting molar teeth. A persistent enamel organ at the apex of each tooth provides a constant supply of ameloblasts and cells forming the stratum intermedium. Once enamel production is com-

pleted these epithelial cells persist as islands (one row of low columnar ameloblast remnants and one layer of stratum intermedium cells) between the attachments for the principal fibers of the periodontal membrane on enamel. These islands of cells are carried upwards as the tooth erupts. Their ultimate fate in normal animals is illustrated in Fig. 1, which is a series of photomicrographs of an interdental septum. Just before reaching the oral epithelium, and some distance above the apex of the interdental bony septum, the cells of the stratum intermedium proliferate and invaginate the underlying connective tissue. This layer then appears as stratified squamous non-keratinizing epithelium. On reaching the oral epithelium these cells merge with the stratified squamous cells of the oral epithelium.

Deficient Animals No change in the appearance of this layer was noted in animals on 0.4 mgs. ascorbic acid daily.

Animals receiving 0.0 mgs. ascorbic acid exhibited marked changes. The cells of the stratum intermedium proliferated at a much lower level on the tooth surface (Figs. 2, 3, 4). Proliferation was more extreme just above the level of the interdental bony septum. It was a proliferation of stratum intermedium cells already existent in the area and not a proliferation of oral epithelium downwards along the tooth surface.

C. Bone

(a) Normal animals.

This study in normal animals has revealed that the guinea pig molar teeth not only erupt continuously but they also grow wider in diameter as the animal ages. This increase in tooth size was reflected in a typical bone deposition, bone resorption pattern which was found in histological sections of all normal animals studied in this experiment. Schematic diagram 1 of a cross section of the four molar teeth illustrates the following:

First molar.

1. The mesial border of the tooth remained in a fixed position.
2. The increase in diameter of the tooth caused resorption at the mesial and buccal bone margins of the alveolus.

3. Bodily movement of the tooth in a buccal direction resulted in bone deposition at the lingual bone margin of the alveolus.

Second molar.

1. This tooth expanded buccally and distally.
2. It moved bodily in a buccal and distal direction.

Third molar.

1. The third molar expanded distally and buccally.
2. No bodily shift in a buccal direction was noted.

Fourth molar.

1. The fourth molar expanded buccally, distally and lingually.
2. There was no bodily shift in a buccal direction.

Bone apposition was also seen on buccal, lingual, and mesial, external surfaces of the mandible.

(b) Deficient animals.

1. In OVB, the group on 0.4 mgs. ascorbic acid for 75 days, five of the seven animals showed resorption of bone at the lingual bone margin of the alveoli of the second and third molars. Resorption was not seen in this location in normal animals.

2. In I, the group on 0.0 mgs. ascorbic acid for 24 days, a generalized bone resorption was superimposed on the pattern seen in normal animals, i.e. all bone margins of the alveoli exhibited resorption, but this was more pronounced in areas of pressure.

D. Periodontal Fiber Bundles

The collagen fiber bundles of the periodontal membrane are constantly being formed, rearranged, and finally dissolved in continuously erupting teeth.

(a) Normal animals.

The principal fibers form and are cemented to the newly completed enamel by means of small deposits of cementum called 'cement pearls'. The fiber bundles are carried occlusally with the tooth and disappear just before reaching the oral epithelium.

(b) Deficient animals.

1. Animals receiving 0.4 mgs. for 48 and 75 days respectively, showed no change in this pattern.

2. Sections from animals receiving 0.0 mgs. ascorbic acid for 24 days revealed that the heavy collagen bundles of the periodontal membrane did not form. Fibers which had been formed prior to the deficiency were still to be seen just below the level of the oral epithelium.

E. Dentin

Severe changes in the dentin formed during the experimental period were seen in Group I (0.0 mgs. ascorbic acid). In the last days of the experiment the width of the dentin formed was very much reduced and the dentin formed was not regularly arranged. Odontoblasts were surrounded by ground substance that stained much like cartilage. Dentin with a regular arrangement of tubules, formed before the deficiency, was covered at its inner surface by this homogeneous dentin. Animals in group IVA and IVB (0.4 mgs. ascorbic acid for 48 and 75 days) formed a similar type of dentin with odontoblasts surrounded by matrix. Molar teeth of animals in group IVA (48 days) still had some normal dentin at the occlusal surface while in group IVB (75 days) the disturbed dentin was seen to encompass all of the dentin. Animals in group V (0.4 mgs. ascorbic acid, 48 days, then 5.0 mgs. ascorbic acid, 27 days) had formed abnormal dentin at first and normal dentin during the latter days.

These changes were similar in appearance to those described by previous experimenters.

F. Bone Resorption at the Growing Ends of the Molar Teeth.

(a) Normal animals.

Bone resorption was noted at the fundus of the molar teeth of all normal animals. Bone apposition was seen at the lower border of the mandible.

(b) Deficient animals.

In sections from animals in group IVA and IVB (0.4 mgs. ascorbic acid), no quantitative difference from the appearance of bone apposition or bone resorption in normal animals could be detected. Animals in group I (0.0 mgs. ascorbic acid) showed increased bone resorption at the fundus and lack of bone formation at the lower border of the mandible.

Discussion

Other experimenters, Fish and Harris (2), have noted bone resorption on one side and bone deposition on the opposite side of the interdental boy septum between guinea pig molars. They have correctly assumed that this is an indication of tooth movement. However, previous reports in the literature do not explain that this tooth movement is caused partly by increase in tooth size, and the precise pattern of movement has not previously been defined. The detailed description of tooth movement made possible by the present investigation should be of value in evaluating minute deviations from normal under various experimental conditions.

Changes in dentin of the molar teeth of all deficient animals compare favourably in appearance with changes described in previous experiments. These typical changes, together with differences indicated on weight graphs 1, 2 and 3, serve as evidence that deficiencies of ascorbic acid did in fact exist.

It was evident from this study that during the time the animals were in a severe deficiency state, collagen bundles did not form in the periodontal membrane. It is suggested that the increased proliferation of stratum intermedium cells, observed at a lower level

on the side of the tooth, is a secondary effect of the deficiency, i.e. stratum intermedium cells, like the basal cells of any stratified epithelium, retain the potential for proliferation. They do proliferate in this instance because normal connective tissue of the periodontal membrane was not present to confine them.

Sicher (3) has described a 'hammock ligament' at the apex of all continuously erupting teeth. He suggested that pressure from the expanding pulp was transmitted to the hammock ligament, and from there to the bone on the lateral walls of the alveolus as a beneficial pulling force. They have stated that the presence of this ligament prevented resorption of the underlying bone. However, in this experiment it was found that while reticular fibers outlined the area described, there were no collagen fibers present that would constitute a ligament of sufficient strength to transmit forces to the socket walls. Indeed, resorption of the bone was found at the apices of the teeth of all normal guinea pigs. Bone apposition at the lower border of the mandible apparently prevents the bone from eroding through in this region. It is suggested that if there is any downward pressure due to the expanding pulp, it is not entirely utilized as an eruptive force, but some of the energy is expended as resorptive pressure on the underlying bone.

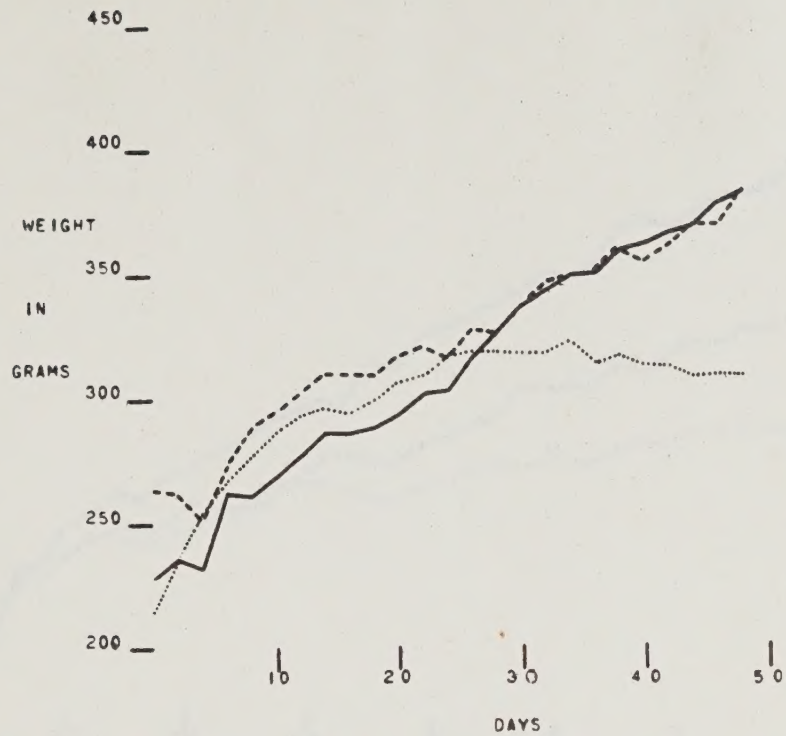
Material from this experiment is being assembled at present and will be submitted in the near future as a thesis in partial fulfillment of the requirements for the degree of M.Sc.D.

Bibliography

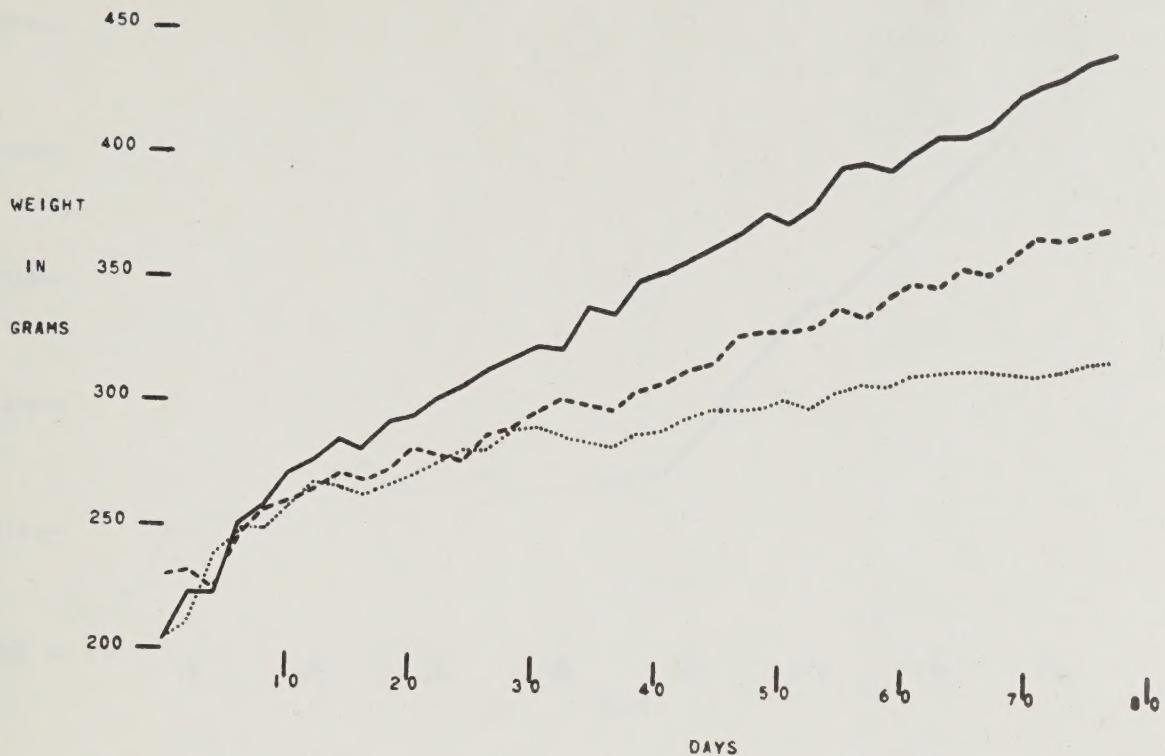
1. Reid and Briggs, Synthetic Diets for Guinea Pigs. J. Nutrition, 51: 341, 1953; 52: 507, 1954.
2. Fish and Harris, Brit. Dent. J. 58: 3, 1938.
3. Sicher, Oral Anatomy Text, 1949.

Table 1. Distribution of guinea pigs according to type of feeding, amount of ascorbic acid, and length of time on experimental feeding.

Group	No. of animals	Ascorbic acid per day per animal	No. of days on diet	Type of feeding
I	5	0.0 mgs.	24	ad lib.
IIA	6	5.0 mgs.	48	" "
IIB	9	5.0 mgs.	75	" "
IIIA	6	5.0 mgs.	48	pair fed to IVA
IIIB	7	5.0 mgs.	75	pair fed to IVB
IVA	6	0.4 mgs.	48	ad lib.
IVB	7	0.4 mgs.	75	" "
V	5	0.4 mgs.	48	" "
		then 5.0 mgs.	27	" "



GRAPH 1. DAILY WEIGHT RECORDS OF
GROUP IIA (5.0 MG. ASCORBIC ACID, AD LIB) _____
GROUP IIIA (5.0 MG. ASCORBIC ACID, PAIR FED
TO IVA) -----
GROUP IVA (0.4 MG. ASCORBIC ACID, AD LIB.



GRAPH 2. DAILY WEIGHT RECORDS OF
GROUP IIB (5.0 MG. ASCORBIC ACID, AD. LIB.)
GROUP IIIB (5.0 MG. ASCORBIC ACID, PAIR FED
TO IVB)
GROUP IVB (0.4 MG. ASCORBIC ACID, AD LIB.)

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L



GRAPH 3. DAILY WEIGHT RECORDS OF

GROUP V. 0.4 MGS. ASCORBIC ACID, 48 DAYS,
THEN 5.0 MGS. ASCORBIC ACID, 27 DAYS,

.....

L

JUNCTION WITH STRATIFIED SQUAMOUS
ORAL EPITHELIUM

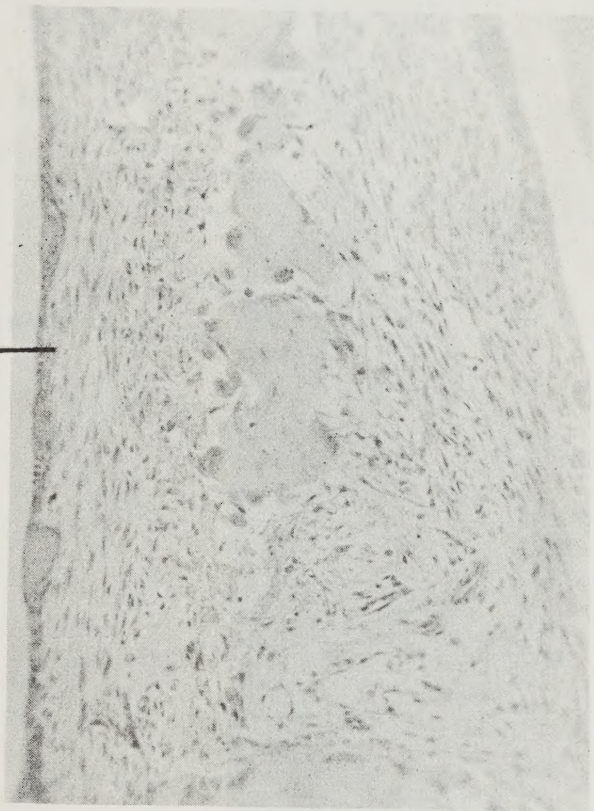
STRATUM INTERMEDIUM CELLS BEGIN TO
PROLIFERATE

ONE LAYER OF REDUCED AMELOBLASTS
ONE LAYER OF STRATUM INTERMEDIUM

APEX OF INTERDENTAL BONY SEPTUM

FIG. 1. L.P. PHOTOMICROGRAPH. GROUP 11B (5.0 MGS. ASCORBIC ACID).
PERIODONTAL MEMBRANE IN INTERDENTAL SPACE ABOVE APEX OF
BONE MARGIN.

PROLIFERATION OF STRATUM
INTERMEDIUM CELLS BEGINS



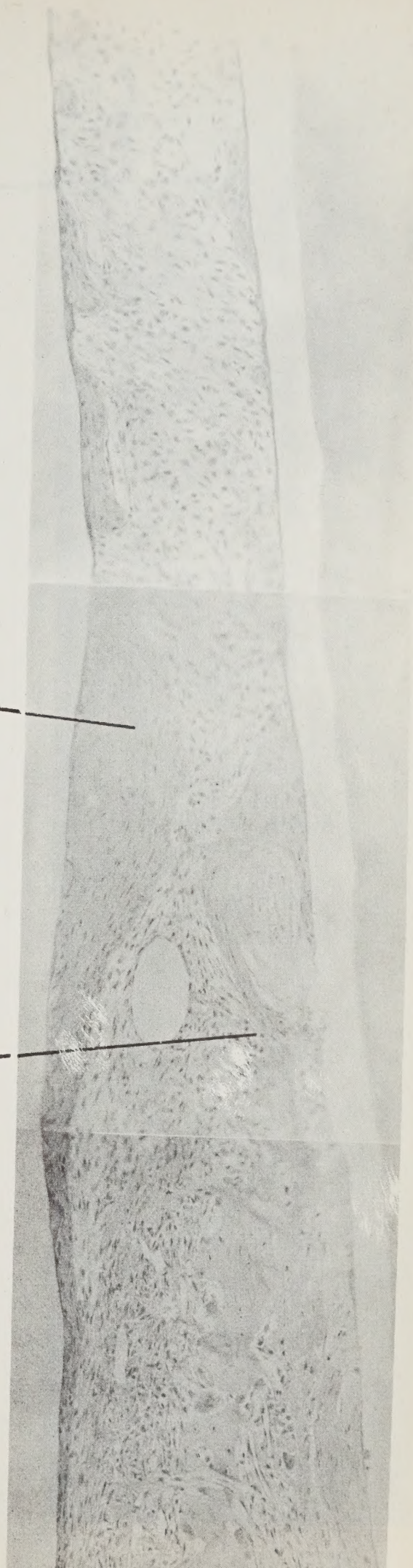
L.P. PHOTOMICROGRAPH.

FIGURE 2. GROUP 1 (0.0 MGS. ASCORBIC ACID).
INTERDENTAL BONY SEPTUM AND PERIODONTAL MEMBRANE.

EXTREME PROLIFERATION OF STRATUM
INTERMEDIUM CELLS JUST ABOVE BONY APEX

APEX OF INTERDENTAL BONY SEPTUM

FIGURE 3 (CONTINUATION OF FIGURE 2)
GROUP 1, 0.0 MGS. ASCORBIC ACID.
PERIODONTAL MEMBRANE IN INTERDENTAL



ORAL EPITHELIUM

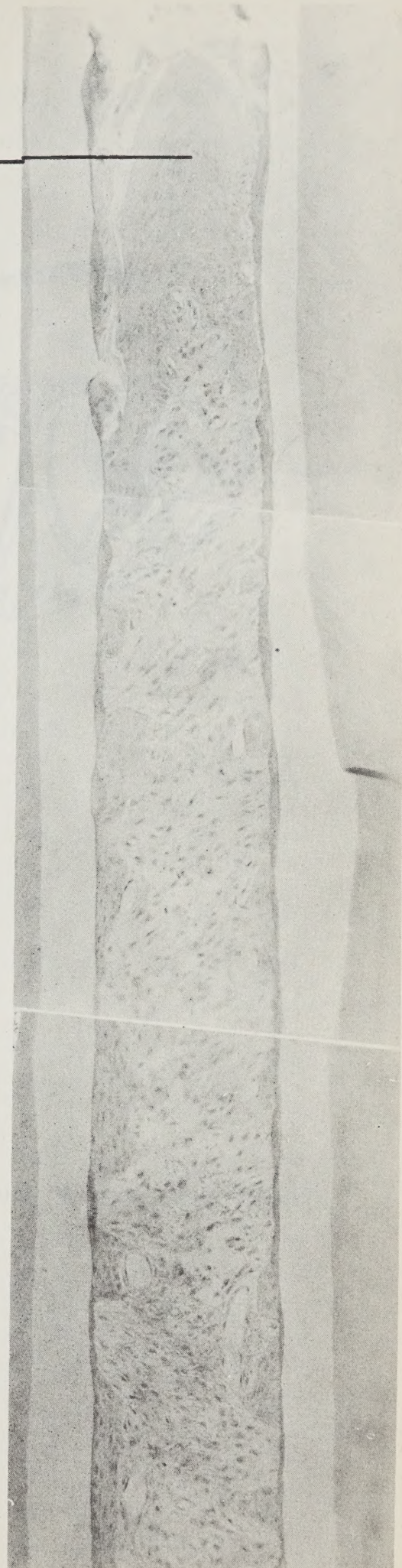


FIGURE 4. (CONTINUATION OF FIGURE 3.)

GROUP I (0.0 MGS. ASCORBIC ACID)

L

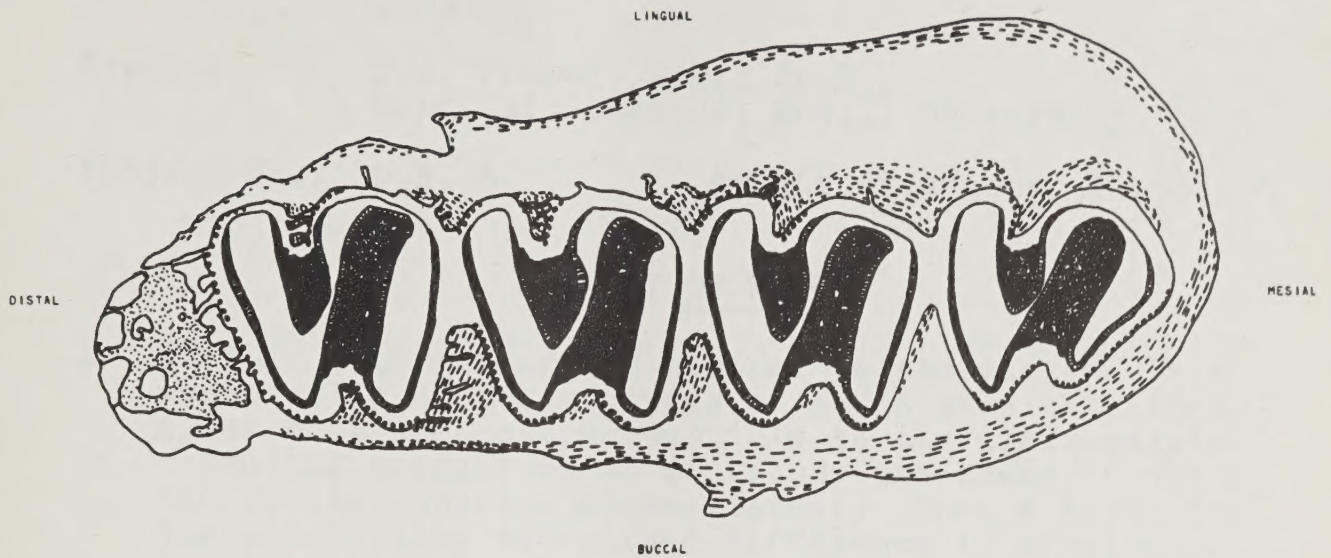


DIAGRAM 1.

CROSS SECTION OF LOWER RIGHT MANDIBLE JUST ABOVE BONE CREST.

LEGEND: BONE DEPOSITION -----

BONE RESORPTION

APPENDIX M

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 1957

Subject: Studies on the pathogenesis of cleft palate.

Grantee: F.C. Fraser, M.D., Ph.D.,
Dept. of Genetics, McGill University.

Project No.: D.R. 60 Amount of Grant: \$1,588.00

SUMMARY OF WORK

1. Further observations on time of palate closure in 4 genetically different types of mouse show that those types that are most susceptible to the cleft-palate-producing properties of cortisone are those in which the palate normally closes latest. Thus a basis for the genetically determined difference in cortisone-susceptibility may lie partly in the genetically determined pattern of normal development.
2. Further support for the above idea is provided by a comparison of embryos from light and heavy mothers of the same genotype. Embryos from heavier mothers have fewer cleft palates than embryos from lighter mothers, following cortisone treatment. The small body of data so far collected indicates that the palates of embryos from untreated heavy mothers begin closing earlier than the palates of embryos from light mothers.
3. Some light has been thrown on the question of why genetically similar embryos within the same uterus react differently to the same teratogen. Cortisone-treated embryos were observed just after the normal time of palate closure, in a cross expected to give an intermediate frequency of cleft palates. In the litters observed very shortly after the normal time of palate closure, the animals with cleft palates tend to be retarded in their general development, but this difference disappears within a few hours. It is not clear whether the cortisone affects more severely the relatively retarded animals, or whether the cortisone retards development of some embryos

(including shelf movement) more than others. At least it can be said that the differences between litter-mates to the cleft-palate producing qualities of cortisone do not reside in the palate alone.

4. Following the discovery that the injection of small amounts of fluid (cortisone vehicle, physiological saline) into the uterine cavity can cause cleft palate in embryos of the treated horn, (see previous report), the effect of needle-puncture alone has been studied. Puncturing the wall of the pregnant uterus with a needle, without injecting anything, does not appear to cause cleft palates in the embryos.
5. The teratogenic effects of a new vitamin-antagonist, 6-amino-nicotinamide, have been studied. Maternal treatment with this substance caused cleft palate in the offspring when treatment was begun 9, 10, 11, 12 or 13 days after conception. The highest frequency appeared to be produced by treatment on day 13, one day before normal palate closure. In addition to the effects on the palate a variety of other congenital defects were observed, including cleft lip, absent or deformed tibiae, absence of pre-axial digits, syndactyly, fusion of vertebrae, malformed sternum, and other minor defects. It thus appears that nicotinic acid deficiency has profound harmful effects on the developing embryo.

PROGRESS REPORT

I. Time of Embryonic Palate Closure

(a) Strain Differences. This work has developed from the discovery (Fraser and Fainstat, 1951) that cortisone, injected into pregnant mice, would cause congenital cleft palate in the offspring, and that there are genetically determined differences in the frequency of cleft palate induced by this treatment.

In a previous report (D.R. 60, March 1956) evidence was presented that indicated a relation between genetic susceptibility to cortisone-induced cleft palate and the normal time of embryonic palate closure. Further material has been collected and the results, presented in Table 1, continue to demonstrate this relationship - mice of genotypes resistant to the cleft-palate producing properties of cortisone have palates that tend to begin closing relatively early in development.

Table 1. Duration of embryonic palate closure
in untreated mice of various genotypes

Cross	% cortisone induced cleft palate*	No. of embryos	Duration of palate closure	
			from days/hrs.	to days/hrs.
A/Jax x A/Jax	100	129	14/18	15/8
A/Jax x C57BL	43	132	14/12	15/8
C57BL x C57BL	18	157	14/8	15/10
C57BL x A	4	132	14/0	14/20

Tables 2, 3, 4 and 5 show the pattern of palate closure plotted against morphological rating. Palate closure is classified into six stages from 1 (completely open) to 7 (completely closed). Morphological rating is based on a number of morphological features to which numerical values have been assigned, and a mean calculated for each embryo. Details of palate stage classification and morphological

* Kalter (1954)

Table 2

A

Palate Stage

	1	2	3	4	5	6	7
-3	11						
-2							
-1							
0							
1							
2	1						
3	1						
4	2						
5	2						
6	8						
7	6						
8	7	3					
9	3						
10	2	2	2	1	1	6	1
11	1		3	2		7	5
12						2	7
13					1		23
14							8
15							2
16							5
17							1
18							1
19							2

Table 3

A x B

Palate Stage

	1	2	3	4	5	6	7
-3							
-2							
-1	1						
0	1						
1	1						
2							
3	2						
4	19						
5	7						
6	3						
7	3		1				
8	7	1	5	3	3	5	
9			1	1		10	
10			1			5	4
11						5	6
12						2	13
13							9
14							3
15							6
16							2
17							2
18							
19							

Morphological Rating

Table 4

Table 5

Morphological Rating	B x A							B						
	Palate Stage							Palate Stage						
	1	2	3	4	5	6	7	1	2	3	4	5	6	7
-3								-3						
-2								-2	4					
-1	1							-1	2					
0	2							0	6					
1	6							1	2					
2	3							2	2					
3	6							3	2					
4	7	1						4	37	3				
5	4	1						5	1	1	1	1		
6	2			1				6	1	2	1	2		
7	4	2	1		7	2		7		1	5		3	
8	3			7	2	11		8		2	5	4	10	1
9					1	12		9		1		1	2	5
10						8	5	10		1			7	1
11						3	8	11					3	8
12							12	12						9
13							8	13						9
14							2	14						6
15								15						2
16								16						1
17								17						
18								18						
19								19						

ratings have been published by Walker and Fraser (1956). Again the impression appears in Tables 2 and 5 that palates in strain A/Jax (referred to as "A") which is susceptible to cortisone, begin to close at a later stage of embryonic development than palates in strain C57BL (referred to as "B"), which is resistant to cortisone. Similarly (Tables 3 and 4) in the more resistant type of F1 hybrid (C57BL x A/Jax, referred to as BA) the palates appear to begin closing at an earlier stage of development than in the more susceptible type (A/Jax x C57BL, referred to as "AB"). If this trend is a real one, the palates of the C57BL x A hybrids might be expected to close a little earlier than those of the C57BL animals, but all that can be said of the data at present is that the distributions are similar, and that if a small difference does exist it may well have been obscured by the variability of the material.

Conclusion. The results continue to support the idea that embryos in which the palate closes early are more resistant to the cleft-palate producing effects of cortisone than those in which the palate closes later.

(b) Time of palate closure and maternal weight. Previous work in this laboratory (Kalter, 1955) has shown that the cleft palate frequency in the offspring of cortisone-treated backcross females which are young and light in weight is significantly high than in the offspring of old heavy females.

If the conclusion reached in the previous section is true, then light mothers should have embryos with later palate closure than heavy mothers. To test this, F1 females of the crosses A/Jax x C57BL (called "AB") and C57BL x A/Jax (called "BA") have been raised. These have been backcrossed to A males either at puberty when they are light or after 4 months of age when they are heavier. The female weights were recorded on the day a vaginal plug was observed.

At present litters from 30 females have been examined. Time of palate closure of embryos from AB mothers does not appear to differ from BA mothers. This is consistent with earlier experiments (Kalter, 1954) where the cortisone induced incidence of cleft palate for the AB x A cross (22%) did not differ significantly from the BA x A cross (25%). Therefore the 30 litters from AB or BA females have been divided in half so that palate

closure time of embryos from the 15 lightest females (Table 6) can be compared with those from the 15 heaviest females (Table 7). At present there is a very slight indication that the palates of embryos from heavy (resistant) mothers start to close earlier (Table 7, rating 7) in morphological development than those from light (susceptible) mothers (Table 6, rating 8). When palate stage is plotted against chronological age embryonic palates from heavy mothers start to close at 14/0 (14 days 0 hours) while those from light mothers start to close at 14/4, with the end of palate closure for both groups being at about 14/16. No statistical analysis has been attempted at this early stage of the investigation.

Conclusions. The idea that embryos from light mothers should have later palate closure than those from heavy mothers has gained some support. The results so far indicate that palate closure appears to occur slightly earlier in morphological as well as chronological development of embryos from heavier mothers.

II. Morphological Development of Embryos with and Without Cleft Palates.

A problem about which we are almost completely ignorant is the question of why some embryos in a given uterus get cleft palates following a given stimulus and others do not. Perhaps it is because the relatively retarded embryos are more susceptible to teratogenic insults. To test this a comparison is being made, within litters from cortisone treated females, of embryos with cleft palate versus their non-affected litter mates as to morphological rating.

F1 females of the A x B and B x A cross have been raised. They were mated with A males, treated with cortisone in the standard manner and sacrificed late on day 14 and early on day 15. The results so far are summarized in Table 9. The litters have been placed in ascending order of chronological age. In the earliest litter examined (litter 1) 9 of the 10 embryos had cleft palate. The Mean Morphological Rating (MMR) of those with cleft palate (15.89) was lower than that of the embryo with closed palate (20.00). In litter 2 the difference between the MMRs could be tested. Here the MMR of embryos with cleft palate (13.0 ± 0) was significantly lower than that of embryos with closed palate (20.19 ± 0.9916).

Table 6

Light
Palate Stage

1 2 3 4 5 6 7

-3 -3

-2 -2

-1 -1

0 0

1 1

2 4

3 12

4 12

5 4

6 3

7 7

8 1

9 1 3

10 1 2 8 1

11 7 4

12 2 8

13 3

14

15

16

17

18 1

19

20 1

21 3

Table 7

Heavy
Palate Stage

1 2 3 4 5 6 7

-3 -3

-2 -2

-1 1 1

0 2 2

1 1 1

2 2

3 7

4 15

5 3

6 7

7 9 1

8 6 1 1

9 1 1 3

10 1 1 11

11 8 5

12 7 2

13 10

14 5

15 4

16

17 3

18 1

19

20

21

Morphological Rating

Table 8

Comparison of mean morphological ratings of embryonic litter mates with cleft palate versus closed palate. Mothers were AB or BA crossed to A males and treated with 2.5 mg cortisone on 4 successive days starting day 11.

Litter	Mother	Chron. age days/hrs.	No. cleft palate	cleft	Mean Norph. Rating (MMR)	P value
1	AB	14/20	9/10	15.89	20.00.	
2	BA	14/22	2/10	13.00 ± 0	20.19 ± 0.9916	<.001
3	BA	15/0	6/11	15.92 ± 1.8876	18.10 ± 1.8534	.5-.4
4	AB	15/0	6/11	28.17	28.60	
5	BA	15/2	4/6	25.50	26.75	
6	AB	15/2	8/11	27.56	27.83	
7	AB	15/2	3/10	28.00	27.86	
8	AB	15/4	7/10	28.79 ± 0.5791	30.00 ± 0	.1-.05
9	AB	15/6	2/7	28.5	27.8	
10	BA	15/8	2/10	28.17	28.71	
11	AB	15/10	6/11	28.92	28.75	

None of the differences between MMRs in the remaining litters were statistically significant. All that can be said at present is that in litters up to 15/4 (litter 8) the MMRs of embryos with cleft palate have a tendency to be slightly lower than the MMRs of their closed palate litter mates. This is no longer the case in the older litters (9, 10 and 11).

Conclusions: These preliminary results have shown that there may be a tendency for embryos with cortisone-induced cleft palate to be more retarded than their closed palate litter mates, at least for a short time after palate closure time. To investigate this further more litters in the day 14/20 to 15/0 range will be collected and examined.

III. Cleft Palate Caused by Intra-uterine Injections

(a) Amniotic sac puncture. This work has now been published (Trasler et al, 1956).

(b) Extra-amniotic intra-uterine injections. The discovery that cortisone vehicle (without cortisone) when injected into the uterine cavity, could cause cleft palates in the embryos led us to test the effects of injecting other materials. As previously reported, pregnant females were etherized on day 12 or 13 of gestation, the abdomen was opened, and a needle inserted into the uterine cavity, care being taken not to puncture the amniotic sac. After the injection, the animal was sewn up, and killed on day 18 for examination of the embryos. Results for injection of cortisone, cortisone vehicle, and physiological saline have been presented in a previous report and are included here only for comparison.

As previously reported (D.R. 60, 1956), it was found that the injection of 0.10 cc cortisone vehicle (without cortisone) caused 4/20 cleft palates and also that physiological saline caused 3/32 cleft palates. The question then arose "Are the defects due to the presence of fluid in the uterine cavity, or could they be due to uterine irritation, caused by the needle puncture".

The effect of inserting a needle without fluid into the uterus has therefore been studied. In this

Table 2

Day	Treatment	No. ♀♀ operated	No. litters aborted	No. litters examined	No. cleft palates treated, horns	No. cleft palates control horns
d13	1.25 mg cortisone	3	0	3	0/9	0/4
d13	2.5 mg cortisone	18	10	8	6/25	2/10*
d12	2.5 mg cortisone	4	1	3	0/9	0/7
d13	cortisone vehicle	8	1	7	4/20	0/12
d12	cortisone vehicle	1	0	1	0/5	0/1
d13	physiol. saline plus phenol	1	0	1	0/2	0/3
d12	physiol. saline plus phenol	1	0	1	0/4	0/3
d13	physiol. saline alone	9	1	8	3/32	0/15
d13	distilled water	11	0	11	0/48	0/17
d13	needle only	50	11	39	1/111	0/65

* These two cleft palates occurred in a litter where there were 5 embryos in each horn and it is possible that the horn recorded as control was treated.

series a needle was inserted into the uterus in the same manner as for injection but no fluid was injected. The results (bottom of Table 9) are 1/111 cleft palates for treated horns and 0/65 for control horns. The one animal with a cleft palate was noted as being small at the time of operation and the needle was not inserted anywhere near it. It may have been a spontaneous cleft palate (the spontaneous frequency of cleft palate is 0.3%, Trasler et al (a), 1956). Thus it is concluded from this that in the great majority of cases it is not the operation or needle puncture per se that causes the clefts, but the fluid.

Since it is felt that the osmotic pressure of the fluid may be of importance (Physiological saline is probably hypotonic to amniotic fluid) the effect of injecting distilled water is being studied at present. So far 11 females have been operated and injected with .10 cc distilled water in the standard manner. These have given 0/48 cleft palates for treated horns and 0/17 for control horns.

IV. Teratogenic Effects of 6-Aminonicotinamide

Through the kindness of Dr. J. D. McColl we were able to begin an investigation of the teratological effects of a recently synthesized vitamin antagonist, 6-aminonicotinamide (Johnson and McColl, 1955). So far the experiments have been exploratory in nature, designed to discover whether this substance does have teratogenic effects, their general nature, and the effective dose range.

(a) Methods. Animals were raised in the Department of Genetics Mouse Colony, on a diet of Purina Laboratory Chow, supplemented once a week with bread, milk and lettuce. Water was available ad libitum. Gestation time was estimated from the time of observation of a vaginal plug, and pregnant females were placed on a wire screen just before delivery so the new-born animals fell through the meshes and were not eaten by the mother. New-born young were observed for gross anomalies and then fixed in Bouin's for histological study, or in 95% ethyl alcohol following which they were macerated in 1% KOH and stained with alizarin red for skeletal study.

50 mg. of crystalline 6-aminonicotinamide was dissolved in 10 ccs. of water and the appropriate

quantity of this solution was injected intramuscularly, in the flank.

(b) Teratogenic dose-range of 6-aminonicotinamide.

In this exploratory stage of the work the approximate limits of the teratogenic dose-range have been established, using cleft palate as an indicator of teratogenicity.

Table 10 shows that in mothers weighing 27 gm. or more on D10 of gestation, single doses of 0.5 - 0.75 mg. were effective in causing cleft palate, whereas doses of less than 0.5 mg. were not. On the other hand, mothers weighing less than 27 gm. on D10 tended to resorb their litters at doses of over 0.5 mg., and did produce young with cleft palates at doses of 0.5 mg. or less. Thus it seems that maternal weight is a much more critical factor in this situation than it is when cortisone is used as the teratogen, and in future doses should be measured in mg. drug/gm. maternal weight.

Conclusion. Single doses of 6-aminonicotinamide given to pregnant mice between the eleventh and the thirteenth day of pregnancy (inclusive) can cause cleft palates in the offspring, the frequency depending on dosage and maternal weight.

(c) Types of congenital defects produced by 6-aminonicotinamide. The cleft palates induced by maternal nicotinamide deficiency resemble, on gross examination, those caused by maternal treatment with cortisone. Detailed embryological and histochemical comparisons have not as yet been made.

There was some indication that the frequency of congenital cleft lip and palate was increased by maternal treatment with 6-aminonicotinamide. In the treated group there were 14 affected out of 92 offspring (15%) as compared to 4 affected out of 59 (7%) in a control group. The difference was not statistically significant, and more data will have to be obtained.

A variety of limb-defects were observed, particularly in the hind-limbs. These included complete or partial absence of the tibia or (rarely) the radius, or hypoplasia and deformity of these bones. It was

Table 10. Frequency of resorption of litters and of cleft palate (CP) in the offspring of pregnant female mice treated with single doses of 6-aminonicotinamide on D10, D11 or D12 of gestation.

Maternal weight	Dose	No. of females	No. of resorpt.	No. of young	No. with CP	% CP
27 gm. or over	over 0.5 mg.	14	2	35	19	54
	0.5 mg.	14	4	25	1	4
	less than 0.5 mg.	9	1	42	0	0
less than 27 gm.	over 0.5 mg.	2	2	0	-	-
	0.5 mg.	19	10	17	9	53
	less than 0.5 mg.	12	4	22	5	23

interesting that in cases of partial absence it was always the proximal segment that was missing. The effective time of treatment was D9, D10 or (rarely D11 of gestation).

Associated with the above defects, or sometimes without them, digital anomalies were often observed. These included absence of the first digit (hallux) and sometimes of the second digit, syndactyly, and absences or synostoses of the metatarsal bones. Sometimes the foot was completely missing.

Vertebral column defects included fusions of the neural arches of sacral or caudal vertebrae with accompanying absence or deformity of the vertebral bodies. The sternum was sometimes bifid (retaining its embryonic condition) or misshapen, with fusions of sternabrae.

Conclusion. 6-aminonicotinamide is a potent teratogen, interfering with pre-skeletal development in many areas of the body, and leading to a wide variety of congenital skeletal defects.

V. Publications

During the year the following papers arising from the work of this project have been published:

Fraser, F.C. Thoughts on the etiology of clefts of the palate and lip. Acta Genet. Statist. Med. 5: 358-369, 1955★.

Trasler, D.G., Clark, K.H., and Fraser, F.C. No cleft palates in offspring of pregnant mice given cortisone after fetal palate closure. J. Hered. 47 (2): 99-100, 1956.

Trasler, D.G., Walker, B.E., and Fraser, F.C. Congenital malformation produced by amniotic-sac puncture. Science 124: 439, 1956.

Walker, B.E., and Fraser, F.C. Closure of the secondary palate in three strains of mice. J. of Embryol. & Exper. Morph. 4: 176, 1956.

★ In spite of the date this issue appeared in July 1956.

VI. References

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- Johnson, W.J., and McColl, J.D. 6-aminonicotinamide - a potent nicotinamide antagonist. *Science* 122: 834, 1955.
- Kalter, H. The inheritance of susceptibility to the teratogenic action of cortisone in mice. *Genetics* 39(2): 185-196, 1954.
- Kalter, H. Modification of teratogenic action of cortisone in mice by maternal age, maternal weight and litter size. *Am. J. Physiol.* 185(1): 65-68, 1956.
- Trasler, D.G., Clark, K.H., and Fraser, F.C. No cleft palate in offspring of pregnant mice given cortisone after fetal palate closure. *J. Hered.* 47(2): 99-100, 1956.
- Trasler, D.G., Walker, B.E. and Fraser, F.C. Congenital malformations produced by amniotic-sac puncture. *Science* 124: 439, 1956.
- Walker, B.E., and Fraser, F.C. Closure of the secondary palate in three strains of mice. *J. of Embryol. & Exper. Morph.* 4: 176-189, 1956.

APPENDIX N

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March, 1957

Subject: Effect of Severe Phosphorus Deficiency on
Bones and Teeth

Grantee: Dr. D. Harold Copp, Dept. of Physiology,
University of British Columbia, Vancouver.

Project: No.: D.R. 59

Amount of Grant: \$5,000.00

SUMMARY OF WORK

In the previous report, it was shown that severe phosphorus deficiency in growing rats produced marked osteoporosis, with extreme demineralization of the bones, while the teeth remained well calcified. There were also differences in distribution of injected radiocalcium.

During the current year, quantitative kinetic studies have been carried out with radiocalcium in which the exchangeable calcium and the accretion rate were measured in femur, tibia, molars and incisors. The total calcium and the accretion rate was greatly reduced in the bones of the deficient animals, although the exchangeable calcium was only slightly reduced. The exchangeable calcium was somewhat lower in the case of the teeth of the deficient animals. However, the accretion rate for incisors was only slightly lower in the deficient animals, and the rate for the molars was the same as for the controls. This may account for the continued growth and mineralization of molars and incisors, even in the presence of severe osteoporosis. Further studies are being carried out to determine whether the resorption rate is increased in severe phosphorus deficiency.

The initial clearance of Ca^{45} and P^{32} from plasma by the skeleton was measured and taken as an estimate of effective bone blood flow. For the period 0-5 and 0-10 minutes in young adult rats, it was found to be 4.8% of the resting cardiac output. It is planned to use this technique to estimate bone blood flow in phosphorus deficient animals, and in specific bones and teeth.

Publication arising from work supported by this grant:

Copp, D.H. Calcium and phosphorus metabolism. Am.J. Med.,
22:275, Feb. 1957.

PROGRESS REPORTINTRODUCTION

Extreme demineralization of the skeleton may be produced by restricting growing rats to a diet very deficient in phosphorus (1). In the preceding report for this project (2), this condition was studied and a progressive loss of calcium and phosphorus from the deficient animals was demonstrated. Phosphorus of the soft tissues appeared to be maintained at the expense of the skeleton, which became extremely demineralized as evidenced by chemical analysis and by x-ray. However, the teeth remained well calcified and the incisors continued to grow and mineralize. Retention of Ca^{45} in bone at 24 hrs. was reduced but the radiocalcium present in molars and incisors was at least as great as in the controls. It appears that the teeth are relatively unaffected by the mineral mobilizing forces which are operating to maintain phosphorus homeostasis in the deficient animals.

During the past year, quantitative kinetic studies have been carried out with radiocalcium, using the techniques of Bauer, Carlsson and Lindquist (3). In addition, experiments have been carried out to determine the initial clearance of Ca^{45} and P^{32} from plasma by the skeleton as a possible means of estimating bone blood flow.

EXPERIMENTAL PROCEDUREExchangeable Ca and Accretion Rate

Female rats of the Wistar strain were weaned at 3 weeks and then restricted to the experimental or the control diet as described in the previous report (2). In the first series there were 15 controls and 15 phosphorus deficient animals; in the second series there were 20 controls and 20 phosphorus deficient. After 34 days on the diet, each rat received a dose of 10 microcuries of Ca^{45} (0.01 mg.Ca) by intraperitoneal injection. The animals were killed in groups at 1, 3, 6, 12 and 24 hrs. after the injection. Samples of blood, muscle, liver, tibia and fibula, femur, tail, incisors, molars, skin and carcass were taken and analyzed for calcium, phosphorus and Ca^{45} . Plasma calcium was analyzed by a modification of the method of Lehman (4), while calcium in the bones, teeth and soft tissues was measured by the Comar (5) modification of the Clark-Collip method (6). Phosphorus was determined by the Sumner (7) modification of the method of Fiske and Subbarow.

Plasma Ca^{45} was determined on 0.2 ml. samples of plasma dried in a stainless steel planchet. Other samples were first dry-ashed in a muffle furnace, dissolved in acid, and aliquots for Ca^{45} determination were prepared, using polyethylene tubes and stainless steel planchets, as described by Comar (5). Suitable corrections were made for mass absorption.

Bone clearance of Ca^{45}

For the determination of Ca^{45} and p^{32} clearance from plasma by bone, adult rats weighing approximately 200-240 gms. were used. They were anaesthetized with urethane and barbitone, and an oil-filled polyethylene cannula was inserted in the carotid artery. This was connected to a micrometric syringe burette powered by a variable speed Palmer kymograph which was adjusted so that blood was withdrawn from the artery at a constant rate over the clearance period. This blood sample was mixed thoroughly. The plasma Ca^{45} concentration in this sample represents the integrated average plasma Ca^{45} concentration over the clearance period. The experimental set-up for withdrawal of blood is shown in Figure 1. The times studied were 0-2, 0-5 and 0-10 minutes. At the end of the clearance period, the rats were killed by cutting out the heart to arrest circulation, and samples of femur, muscle and skeleton were taken. These were analyzed as described above.

RESULTS

The specific activity of Ca^{45} in plasma, muscle and skin are shown in Figures 2 and 3. It is evident that in both groups the skin and plasma rapidly come into equilibrium, indicating a fast movement of calcium between the blood and skin compartments. In the case of muscle, the specific activity remained consistently higher throughout the period studied, possibly because of the slow movement of calcium in and out of the intracellular calcium pool.

The chemical analyses of the bones, teeth and tissues are shown in Table I. The serum calcium was only slightly lower in the deficient group, while the acid soluble phosphorus in plasma was reduced by about 30%. The calcium and phosphorus in femur and tibia was only one fifth the value in the control animals, but the mineral in the incisors and molars was reduced only slightly.

The Ca^{45} present in the various tissues, bone and teeth are shown in Table II. These values have been used to compute the exchangeable calcium and the calcium accretion rate according to the procedure of Bauer, Carlsson and Lindquist using the following formulae:

$$\text{Ca}_{\text{obs}}^{45} = \text{Ca}_{\text{E}}^{45} + \text{Ca}_{\text{A}}^{45} - \text{Ca}_{\text{R}}^{45} \quad (1)$$

$$\text{Ca}_{\text{obs}}^{45} = \text{total amount } \text{Ca}^{45} \text{ present in calcified tissue}$$

$$\text{Ca}_{\text{E}}^{45} = \text{amount of } \text{Ca}^{45} \text{ present in exchangeable fraction of the bone salt}$$

$$\text{Ca}_{\text{A}}^{45} = \text{amount of } \text{Ca}^{45} \text{ incorporated into the non-exchangeable fraction of the bone salt through accretion}$$

$$\text{Ca}_{\text{R}}^{45} = \text{amount of } \text{Ca}^{45} \text{ removed through resorption (or in the incisors of rodents through attrition)}$$

$$\text{Ca}_{\text{E}}^{45} = E \times S \quad (2)$$

$$E = \text{amount Ca } (\text{Ca}^{40} + \text{Ca}^{45}) \text{ in exchangeable fraction}$$

$$S = \text{specific activity of serum Ca}$$

Since it must be assumed that Ca^{45} and Ca^{40} are deposited in the bone in the same proportion as they occur in the blood at the same time we get:

$$\text{Ca}_{\text{A}}^{45} = A \times T \times S_{\text{av}} \quad (3)$$

$$A = \text{rate of Ca accretion}$$

$$T = \text{interval of time between administration of } \text{Ca}^{45} \text{ and observation.}$$

$$S_{\text{av}} = \text{average specific activity of serum Ca during this time interval}$$

Combination of equations (1), (2) and (3) gives:

$$\text{Ca}_{\text{obs}}^{45} = E \times S + A \times T \times S_{\text{av}} - \text{Ca}_{\text{R}}^{45} \quad (4)$$

By knowing the specific activity curve of serum Ca and the total amount of Ca^{45} present in calcified tissue at two or more suitable intervals, A and E can be calculated.

$$\text{Ca}_{\text{lobs}}^{45} = E \times S_1 + A \times T_1 \times S_{1\text{av}} \quad (4:1)$$

$$\text{Ca}_{\text{2obs}}^{45} = E \times S_2 + A \times T_2 \times S_{2\text{av}} \quad (4:2)$$

from these two equations we obtain:

$$\text{Ca}_{\text{lobs}}^{45} - A \times T_1 \times S_{1\text{av}} = E \times S_1$$

$$\text{Ca}_{\text{2obs}}^{45} - A \times T_2 \times S_{2\text{av}} = E \times S_2$$

The exchangeable portions are assumed to be equal and therefore cancel out and it is possible to solve for A. With the obtaining of A it can be substituted in equation (4:1) and E can be solved.

The values for femur, tibia and fibula, molars and incisors are shown in Table III. It will be noted that the accretion rate was very markedly reduced in the bones of the deficient animals. In the case of the incisors, there was a slight reduction in accretion rate, but for the molars, the value is almost identical with that of the controls. The exchangeable calcium is reduced somewhat in the deficient teeth, but is not markedly reduced in the bones. Since osteoporosis is the most striking feature of this deficiency, it is probable that there will be significant changes in the resorption rate, and unfortunately data is not available on this point yet.

Skeletal clearance of Ca^{45} and P^{32}

Bone clearance values were calculated by dividing the rate of uptake by skeleton in % dose/min. by the average plasma concentration in the pool blood sample, expressed as % dose/ml. Since the calcium diffuses rapidly and there is a great excess of exchangeable calcium in bone, it would seem reasonable to assume that Ca^{45} will be completely replaced by non-radioactive Ca by the time the blood leaves the bone blood vessel at the venous end. If this is the case, the plasma clearance computed above will actually be a measure of the bone plasma flow, which in any case can be no less than this value. To express this "estimated bone blood flow" in more tangible terms, it has been expressed as a per cent of the resting cardiac output, using the formula for body surface area of the rat given by Lee (8)

and the value for cardiac index in the rat (2.15 l./min./sq. meter body surface) given by Hoelscher (9). The values are shown in Table IV. Aside from the 0-2 min. clearance period, which is probably not valid since mixing is not complete at that time, there appears to be reasonable consistency between the clearance values obtained. These indicate that the clearance and estimated blood flow through the skeleton is approximately 4.8% of the resting cardiac output (9). This method may have value in determining blood flow through certain specific bones or teeth.

CONCLUSIONS

Previous observations on the effects of severe phosphorus deficiency in growing rats have been confirmed. Kinetic studies have shown a marked decrease in the accretion of calcium by the bones while accretion by molars and incisors was only slightly altered in the deficient animals. There was a slight decrease in the exchangeable calcium in both bones and teeth of the low phosphorus animals, and a very marked reduction in the mineral content of bones. The teeth, however, were well mineralized.

The initial clearance of Ca^{45} and P^{32} from plasma by the skeleton has been used to estimate bone blood flow, which was found to be approximately 4.8% of the resting cardiac output in young adult rats.

(7) Sumner, J.E. A modification of the Fiske and Subbarow method for phosphate analysis. *Science*, 100: 413, 1944.

(8) Lee, M.O. Determination of the surface area of the white rat with its application to expression of metabolic results. *Am. J. Physiol.*, 89:24, 1929.

(9) Hoelscher, B., Effect of adrenalectomy on cardiac output of parabiotic and single rats. *Am. J. Physiol.*, 179:171, 1954.

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- (6) Clark, E.P. and Collip, J.B. A study of the Tisdall method for determination of blood serum calcium with suggested modification. J. Biol. Chem., 63:461, 1925.
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- (8) Lee, M.O. Determination of the surface area of the white rat with its application to expression of metabolic results. Am. J. Physiol., 89:24, 1929.
- (9) Hoelscher, B., Effect of adrenalectomy on cardiac output of parabiotic and single rats. Am. J. Physiol., 179:171, 1954.

Figure 1. Apparatus for collecting integrated blood sample for clearance measurements.

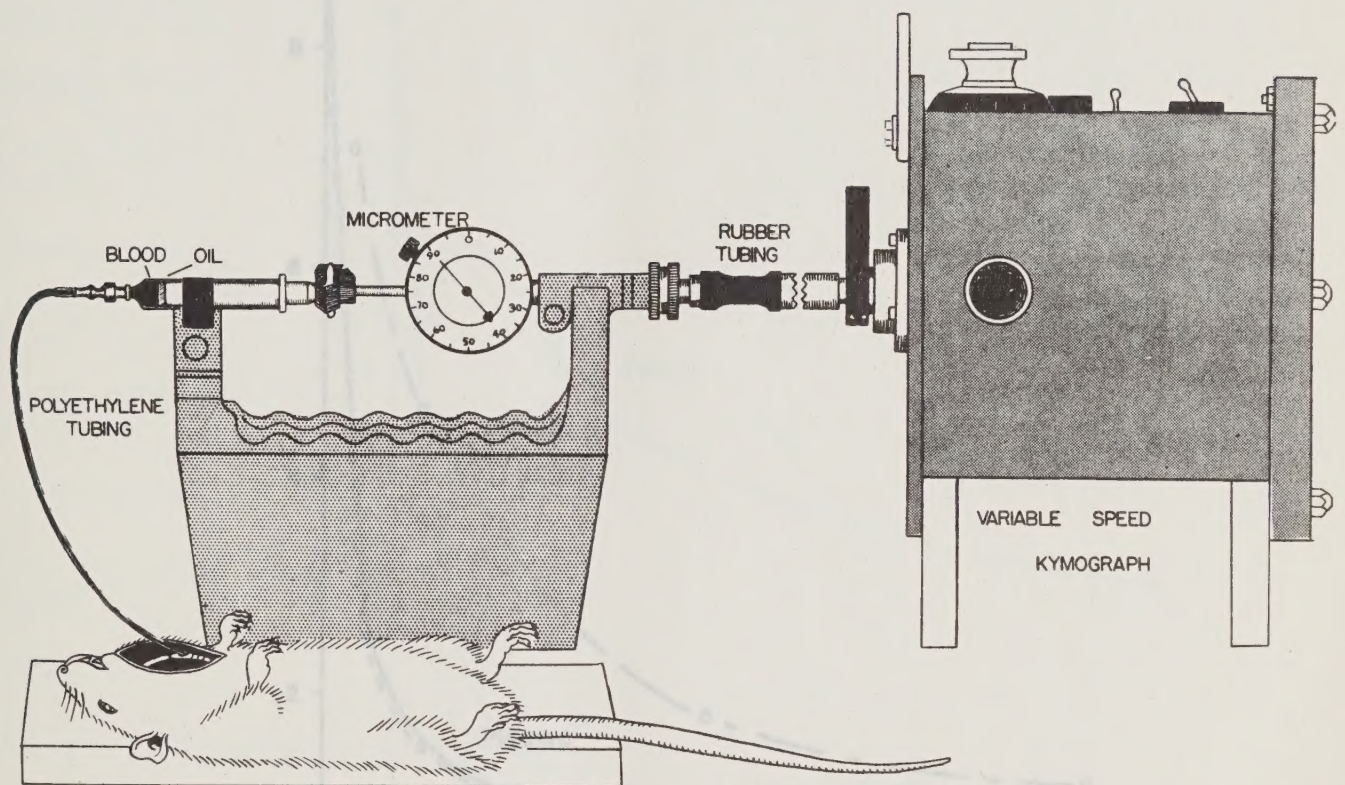


Figure 1. Apparatus for collecting integrated blood sample for clearance measurements.

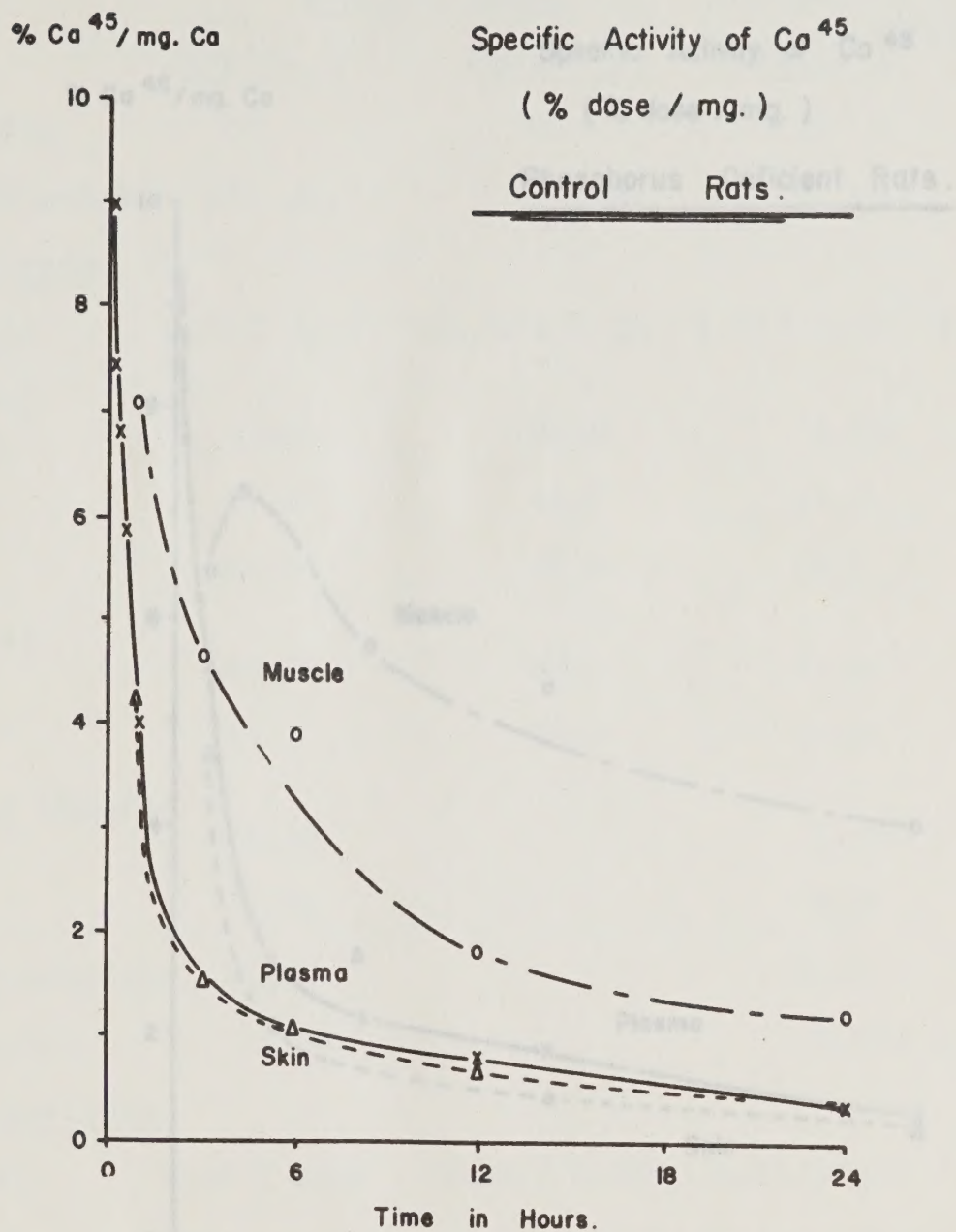


Figure 2. Specific activity of Ca^{45} in soft tissues of control rats.

TABLE I

Calcium and Phosphorus Analyses

Control Group

Phosphorus Deficient

Specific Activity of Ca^{45}
(% dose / mg.)

Phosphorus Deficient Rats.% Ca^{45} / mg. Ca

Number of rats

15

20

Average body weight

133 gm.

Calcium AnalysesPlasma (mg.%)

79.1 ± 0.30

10.23 ± 0.10

8.50 ± 0.54

9.37 ± 0.15

Bones (mg.Ca)

Femur

50.9

30.9

9.23

6.17

Tibia-Fib.

72.8

44.7

11.5

3.7

Tail

72.8

63.8

13.3

9.2

Teeth (mg.Ca)

Molars

74.7

35.1

31.3

29.5

Incisors

77.1

33.9

33.8

33.7

Soft Tissues (mg.Ca)

Skin

23.15

1.51

2.8

Muscle

23.04

19.8

19.8

G.I. Tract + content

17.73 gm.

0.253 gm.

Carbones (mg.Ca)

17.73 gm.

0.253 gm.

Phosphorus AnalysesPlasma (mg.%)

40.1 ± 0.29

10.01 ± 0.30

2.24 ± 0.57

2.24 ± 0.30

Bones (mg.P)

Femur

25.4

34.7

2.8

3.3

Tibia-Fib.

33.4

34.7

2.8

3.3

Tail

33.4

34.7

2.8

3.3

Teeth

Molars

31.0

14.5

8.3

15.7

Incisors

32.3

14.5

8.3

15.7

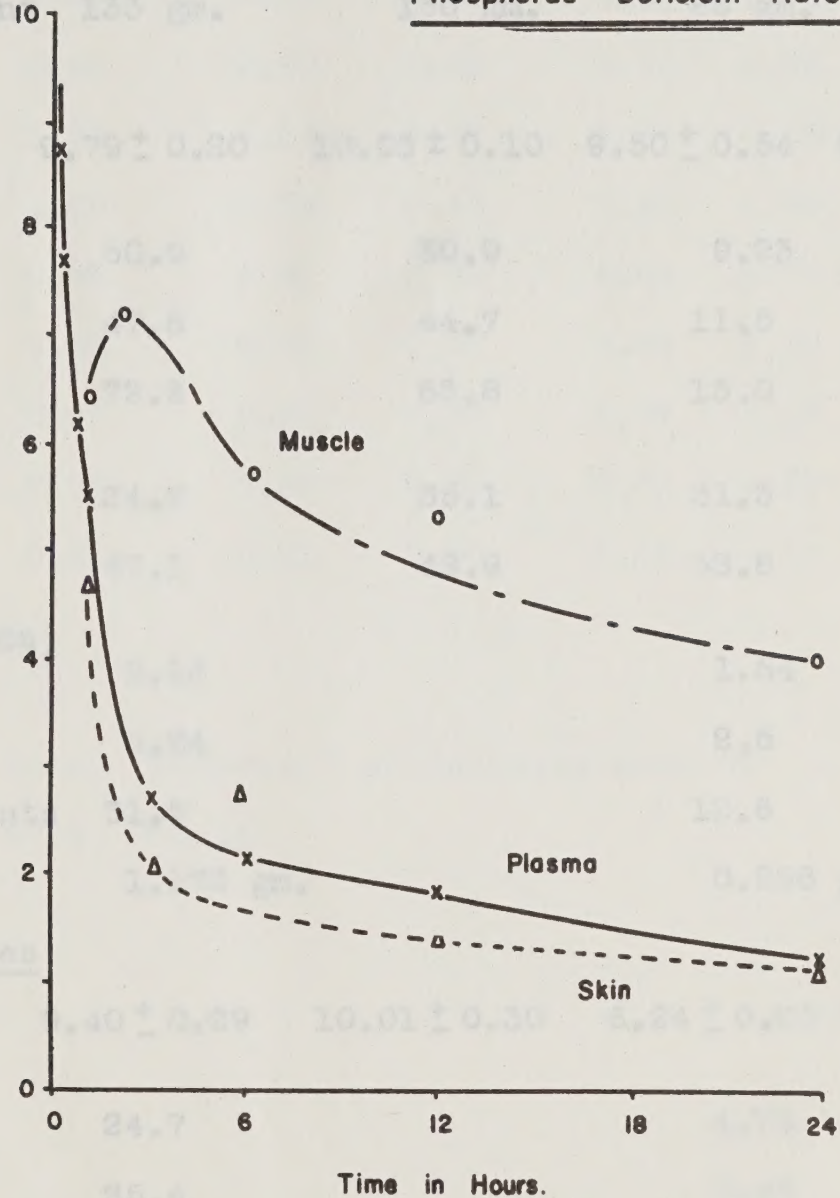


Figure 3. Specific activity of Ca^{45} in soft tissues of phosphorus deficient rats.

TABLE ICalcium and Phosphorus Analyses

	<u>Control Group</u>		<u>Phosphorus Deficient Group</u>	
	<u>Series I</u>	<u>Series II</u>	<u>Series I</u>	<u>Series II</u>
Number of rats	15	20	15	20
Average body weight	133 gm.	150 gm.	85 gm.	91 gm.
<u>Calcium Analyses</u>				
<u>Plasma</u> (mg.%)	9.79 ± 0.20	10.03 ± 0.10	9.50 ± 0.54	9.37 ± 0.15
<u>Bones</u> (mg.Ca)				
<u>Femur</u>	50.9	50.9	9.23	9.17
Tibia-Fib.	47.8	44.7	11.5	9.7
Tail	72.2	63.8	13.0	9.9
<u>Teeth</u> (mg.Ca)				
Molars	34.2	35.1	31.3	29.6
Incisors	47.1	49.9	38.8	36.7
<u>Soft Tissues</u> (mg.Ca)				
Skin	2.13		1.54	
Muscle	3.24		2.5	
G.I.Tract + contents	31.5		12.6	
<u>Carcass</u> (mg.Ca)	1.173 gm.		0.298 gm.	
<u>Phosphorus Analyses</u>				
<u>Plasma</u> (mg.%)	9.40 ± 0.29	10.01 ± 0.30	6.24 ± 0.80	7.04 ± 0.65
<u>Bones</u> (mg.P)				
<u>Femur</u>	24.7		4.74	
Tibia-Fib.	25.4		5.45	
Tail	33.4	34.7	5.8	5.3
<u>Teeth</u>				
Molars	21.0	18.5	18.3	15.7
Incisors	28.2	27.1	21.3	20.7

TABLE II

Radiocalcium in Plasma, Bones and Teeth after Intraperitoneal Injection

Time (hours)	Group	Plasma %dose/mg.Ca	Femur %Dose	Tibia=Fib. %Dose	Tail %Dose	Incisors %Dose	Molars %Dose
1	P def.	5.03	3.07	2.45	2.38	1.07	0.53
	Control	3.54	3.05	2.68	2.63	1.06	0.48
3	P def.	2.55	2.80	2.42	3.33	1.26	0.58
	Control	1.65	3.54	3.00	3.71	1.70	0.48
6	P def.	1.69	2.74	2.45	3.25	1.58	0.58
	Control	0.98	3.51	3.29	4.64	1.77	0.50
12	P def.	1.42	2.81	2.57	2.92	2.16	0.77
	Control	0.66	3.69	3.40	3.87	1.98	0.55
24	P def.	0.95	2.89	2.72	2.61	2.87	0.72
	Control	0.33	3.81	3.62	3.53	2.18	0.47

Average total "recovery" of injected dose of

$$\text{Ca}^{45} = 97.0 \pm 1.1\%$$

TABLE III

Exchangeable Calcium and Calcium Accretion Rate in Bones and Teeth

	Controls	Phosphorus Deficient
<u>Number of Animals</u>	35	35
<u>Average body weight</u>	142 gm.	88 gm.
<u>Femur</u>		
Total calcium Ca	50.9 mg.	9.2 mg.
Exchangeable Ca (E)	1.86 mg.	1.12 mg.
% Exchangeable Ca	3.65%	12.2%
Accretion Rate (A)		
mg.Ca/hr.	0.124 mg./hr.	0.041 mg./hr.
	2.98 mg./dy.	0.98 mg./dy.
<u>Tibia + Fibula</u>		
Total Calcium (Ca)	46.2 mg.	10.6 mg.
Exchangeable Ca (E)	1.55	0.95
% Exchangeable Ca	3.35%	8.97%
Accretion rate (A)		
	0.12 mg./hr.	0.041 mg./hr.
	2.88 mg./dy.	0.98 mg./dy.
<u>Molars</u>		
Total Calcium (Ca)	34.7 mg.	30.5 mg.
Exchangeable Ca (E)	0.29 mg.	0.18 mg.
% Exchangeable Ca	0.84%	0.59%
Accretion Rate		
	0.018 mg./hr.	0.017 mg./hr.
	0.43 mg./dy.	0.41 mg./dy.
<u>Incisors</u>		
Total Calcium (Ca)	48.6 mg.	37.7 mg.
Exchangeable Ca (E)	0.70 mg.	0.24 mg.
% Exchangeable Ca	1.44%	0.64%
Accretion Rate		
	0.077 mg./hr.	0.061 mg./hr.
	1.85 mg./dy.	1.46 mg./dy.

TABLE IV

Skeletal Clearance of Ca^{45} and P^{32} from Plasma in the Adult Rat as an Estimate of Bone Blood Flow

<u>Isotope</u>	<u>Number of Rats</u>	<u>Clearance Period</u>	<u>Skeletal Clearance as % of resting cardiac output</u>
Ca^{45}	9	0-2 minutes	$(6.26 \pm 0.39)^x$
	10	0-5 "	5.07 ± 0.50
	10	0-10 "	4.86 ± 0.38
P^{32}	9	0-5 "	4.49 ± 0.35
			4.81

The main problem centred around the design and construction of a multiplier phototube and circuit used to obtain logarithmic response. The instrument gives linear meter indications for use as direct reading densitometer. Protein concentration of the saliva is measured as a function of optical transmission of the saliva at wave length 270 - 290. At the time the instrument was designed there were no monochromatic filters in the UV range available from scientific supply houses. We thus had to design and construct a monochromatic ultra-violet source by the use of a combination of chemical solution filters which consisted of the following:-

* Report on Fellowship held 1955-56, under the supervision of Dr. A.S.V. Burgen, Dept. of Physiology, McGill University.

APPENDIX O

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Proteins in Saliva

by

Michael Weiss, D.D.S.*

The study of the structure of proteins and their products of metabolism in the digestive secretions in general and in the salivary gland in particular interests to-day not only the physiologists by also the clinician. The protein content, qualitative and quantitative, of a digestive secretion can give an indication of its functional state. Thus a study of the variation of the protein content of salivary secretion can make an important contribution towards the solving of clinical problems in dentistry.

At the start of this project it was felt that the application of electronic instrumentation would be fruitful in facilitating new and more precise observations on this problem. Our objective was the continuous recording of the protein concentration in the saliva by ultra-violet absorption. (Fig. 1), with the simultaneous recording of the flow rate. This would eliminate tedious Kjeldahls on large numbers of samples, which hampered previous investigators in this field. We were hoping this technique would also reveal rapid peaks that might have been previously damped by larger collections.

The main problem centred around the design and construction of a logarithmic photometer consisting of a multiplier phototube and circuit used to obtain logarithmic response to light intensity. The instrument gives linear meter indications for use as direct reading densitometer. Protein concentration of the saliva is measured as a function of optical transmission of the saliva at wave length 270 - 290. At the time the instrument was designed there were no monochromatic filters in the UV range available from scientific supply houses. We thus had to design and construct a monochromatic ultra-violet source by the use of a combination of chemical solution filters which consisted of the following:-

* Report on Fellowship held 1955-56, under the supervision of Dr. A.S.V. Burgen, Dept. of Physiology, McGill University.

- (a) Nickel sulphate (300 grams/litre in water) optical path of 5 cm.
- (b) Corning filter 986 (optical path of 3 mm.).
- (c) Solution of 2,7-dimethyl-3,6-diazacyclohepta-1, 6-diene iodide (synthesized in our laboratory) 20 mg/100 ml. water optical path of 1 cm.
- (d) Carbon tetrachloride pure liquid (optical path of .5 cm.).

A key factor in the instrumentation was to keep the dead space in the cell less than thirty micro-litres. This kept to a minimum the degree to which the sample being measured for protein concentration is diluted by the saliva which preceded or follows, and precludes any measurable damping. In this manner it has been possible for us to record changes in the protein concentration of saliva in terms of short time intervals (i.e. seconds) which to our knowledge has not previously been attempted. A flow meter is connected to the outlet of the cell which responds instantly to the pressure wave set up by the salivary secretion. Both of these measurements were simultaneously recorded in a multi-channel Sanborn recording apparatus.

The experiments were carried out on dog parotid secretion by cannulating Stenson's duct and electrically stimulating the auriculo-temporal nerve. Experiments were also carried out on the submaxillary gland by cannulating the submaxillary duct and stimulating the chorda tympani. Fig. 2 shows a typical Sanborn record, the ordinate of the bottom curve indicates protein concentration and the ordinate of the top curve indicates flow rate. The abscissa indicates the time sequence for both curves. It thus became possible in these experiments with the above apparatus to calculate the protein output of the gland during any instant or period of stimulation. The protein output rate is the product of protein concentration and flow rate. During the first series of experiments it was observed that the protein concentration in the saliva seemed to follow a general pattern after each stimulation. This consisted of two peaks (Fig. 2), the first at approximately fifteen seconds after the start of stimulation and the second at approximately ninety seconds after the start of stimulation.

The series of experiments which followed were designed to elucidate the behaviour of these two peaks in the record of protein concentration in parotid saliva. In these experiments we observed that the protein output in grams in the first peak (P') was dependent upon previous rest interval. Thus when the rate of stimulation was kept constant and the time of rest interval between stimulations varied, it was found that protein output in P' varied directly with the previous rest interval (Fig. 3). We also observed that with a fixed rest interval and repeated stimulation at the same frequency the protein concentration in the second peak (P'') kept dropping but the first concentration peak (P') was unaffected. This implied that the absolute level of P'' was an index of the protein reservoir in the parotid gland which is depleted with continued activity. The protein concentration in P' never dropped below one gram per litre. Fig. 2 is a record at the start of the experiment. The gland has not been depleted and P'' is at a high level of concentration. Figs. 4 and 5 are records at the end of the experiment when after repeated stimulations the gland is depleted of protein. P'' does not differ in these two records. However, P' is different in these two records despite the same rate of stimulation since the rest interval before each of these two stimulations was different. From these facts we put forward the hypothesis that there was a constant stream of serum proteins seeping through into the gland.

We designed several experiments to test this hypothesis. In order to deduce what ratio of the proteins in saliva consisted of serum albumins, we injected I^{131} labelled bovine albumin intravenously and compared the relative radioactivity of the I^{131} labelled albumin in saliva and in arterial blood with varying flow rates and conditions of stimulation. By taking into account the known total serum albumin and I^{131} labelled albumin compared with total protein in saliva and I^{131} labelled protein in saliva we were able to calculate the ratio of serum albumin to total proteins in saliva. From these experiments it appears that in the exhausted dog parotid gland up to fifty percent of the total proteins can be accounted for by serum albumins. We are presently planning experiments with I^{131} labelled gamma globulin and I^{131} labelled lysozyme to observe whether these can account for any of the remaining salivary proteins.

The preceding experiments were followed by similar experiments which were of a preliminary nature on bile and pancreatic juice. It seems from the above preliminary experiments that should the level of serum proteins in the other digestive secretions (i.e. succus entericus, etc.) be of a similar level it would be possible to account for the entire turnover of serum proteins (approximately 5% per day) by secretion in the digestive juices.

Paper electrophoresis was also carried out on dog parotid saliva. We were hoping by this technique to identify the serum proteins appearing in the parotid saliva, and carry through more detailed qualitative studies on salivary proteins. However, thus far we have not been successful in obtaining the number of fractions we expected. We presently postulate that a complex is formed between the mucoproteins in the saliva and the albumins which prevent their separation in the process of paper electrophoresis. This problem is in the process of being clarified by doing paper electrophoresis on parotid saliva after having added I^{131} labelled albumin to parotid saliva. By locating the I^{131} labelled albumin on the paper electrophoresis strip we are attempting to deduce to what extent and between what proteins this complex formation takes place. We are also investigating the formation of complexes between mucoproteins and lysozyme in saliva by bacteriolytic assay of lysozyme using the substrate *Micrococcus lysodeikticus*.

Figs. Va and Vb illustrate data derived from experiments carried out with the intravenous injection of I^{131} labelled albumin to confirm the results of previous experiments on proteins in dog parotid secretion.

Fig. Va illustrates a typical curve of I^{131} labelled albumin activity in parotid saliva after fifteen minutes rest interval from previous stimulation. The ordinate is counts per minute of I^{131} labelled albumin per drop of saliva; the abscissa seconds after the start of stimulation. The first peak in Fig. Va corresponds to P', i.e. the initial peak on the protein concentration curves in the Sanborn records as seen in Fig. II.

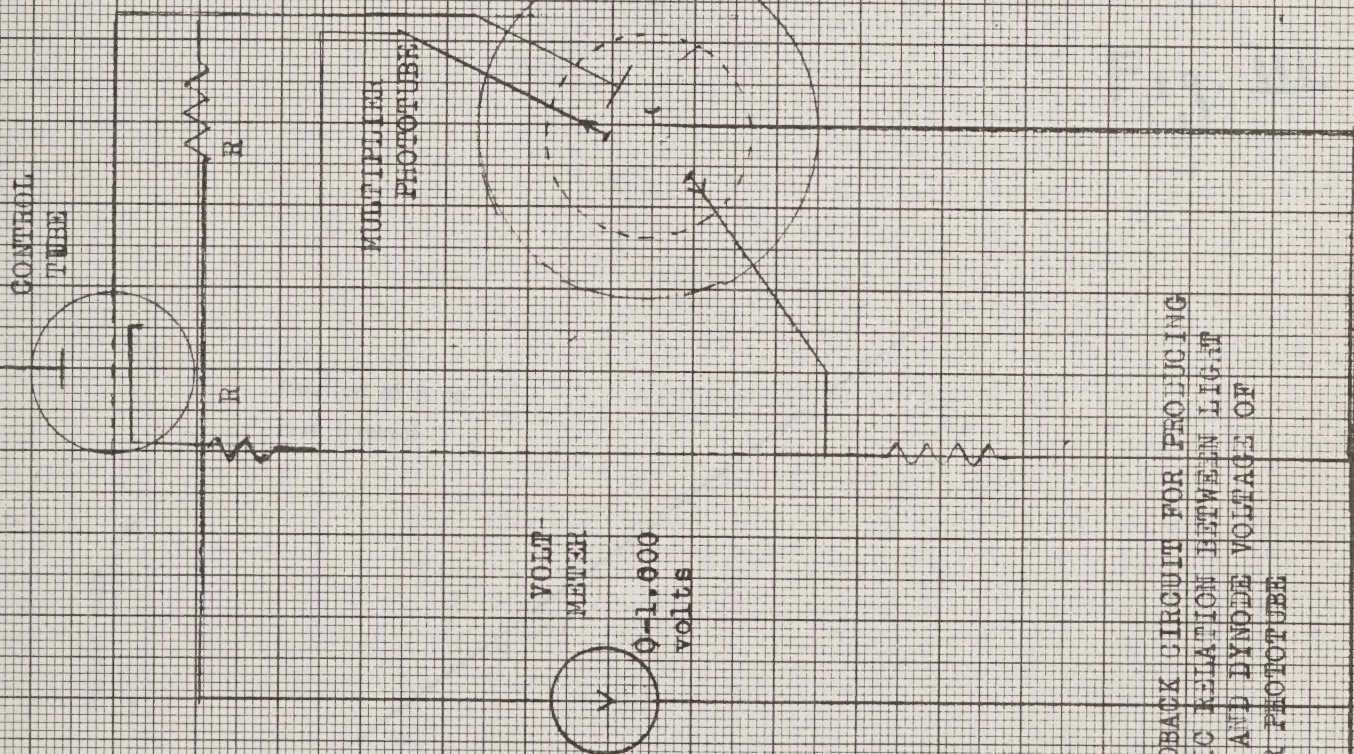
Fig. Vb shows that the amount of I^{131} labelled albumin in the first peak, as indicated in Fig. Va, varies directly

with the previous rest interval. In Fig. Vb the sum of the activities of I^{131} labelled albumin, in counts per minute, in the first peak is the ordinate; the abscissa is minutes of rest interval from previous stimulation. These results seem to confirm our previous hypothesis that there is a constant stream of serum proteins seeping through into the parotid saliva of the dog.

Fig. I

0-6

1200 volts



BASIC FEEDBACK CIRCUIT FOR PRODUCING
LOGARITHMIC RELATION BETWEEN LIGHT
INTENSITY AND DYNODE VOLTAGE OF
MULTIPLIER PHOTOTUBE

SALIVA FROM
FLOW METER

SALIVA FROM
DUCI

LIGHT TIGHT ENCLOSURE
FOR LIGHT FILTERS

ULTRA VIOLET LIGHT
SOURCE

Fig II

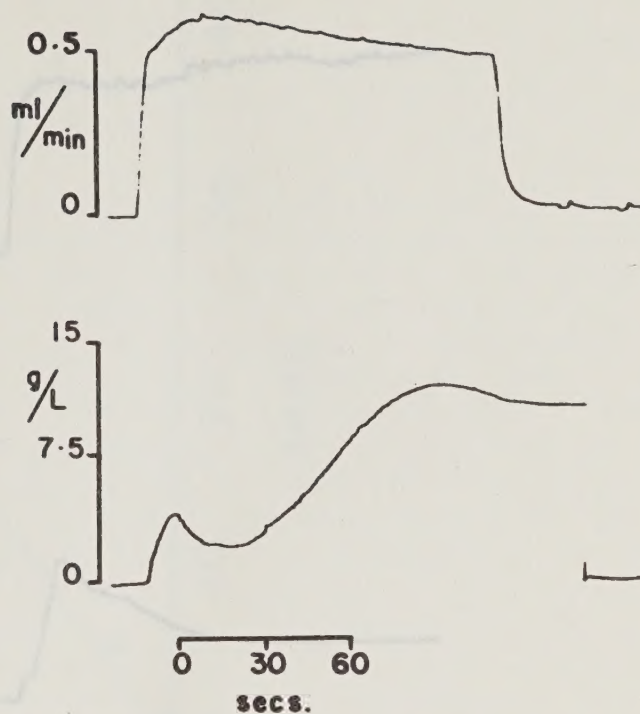


Fig. III

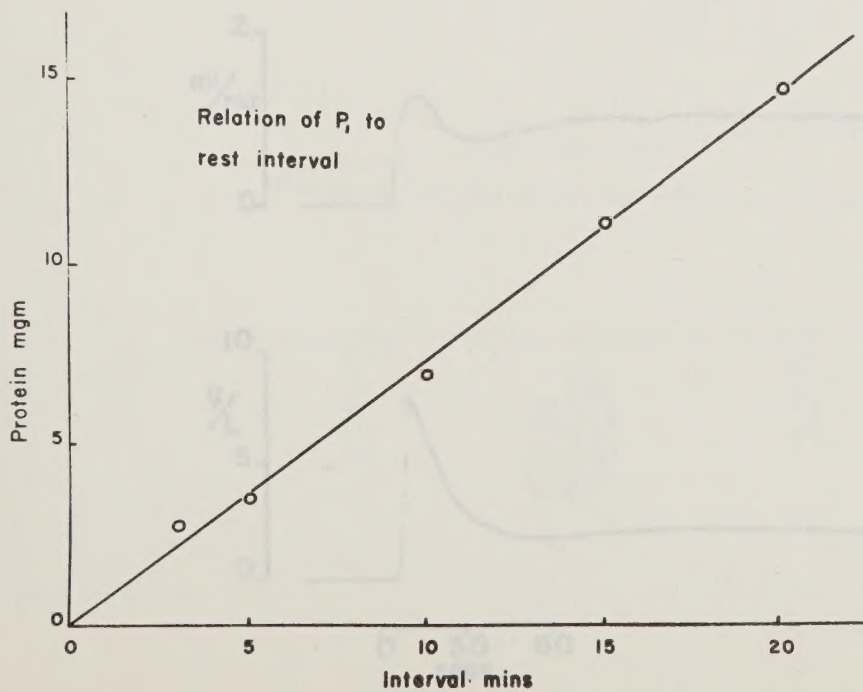


Fig IV

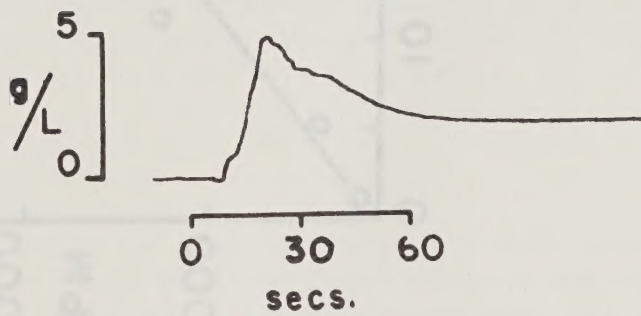
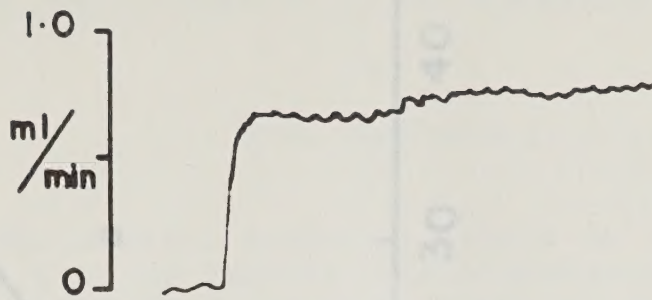
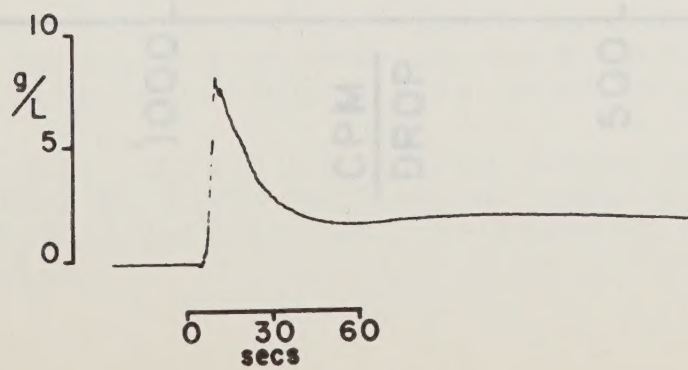
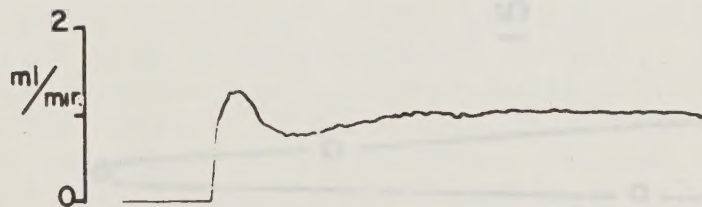


Fig V



SCS

MINI.

APPENDIX P

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Cultural Requirements of Bacteroides nigrescens

by

E.M. Madlener*

The studies reported relate to the growth of Strain K110 of *Bacteroides nigrescens*. Growth of Strain K110 could only be obtained in an agar containing medium in a hydrogen atmosphere. The organism appears to be sensitive to hydrogen in non agar containing media. Growth could only be obtained in agar free media reduced by heat and overlaid with paraffin oil or incubated in a nitrogen atmosphere. The potentiometric behaviour of a medium in a hydrogen and a nitrogen atmosphere was investigated. Growth of K110 appears to be limited to a relatively narrow potential range.

Introduction

The anaerobic strain K110 of the species *Bacteroides nigrescens* is one of the four organisms constituting the pathogenic components of an experimental fusospirochetal infection. This organism requires for growth in artificial media a factor which is produced by one of the three other organisms. *Staphylococcus aureus* and a number of common lab contaminants also produce this growth factor.

Strain K110 has been maintained on blood agar plates cross streaked with *Staphylococcus aureus*. After six days' incubation in Brewer jars a 10 to 20 mm. wide band of black pigmented growth of K110 can be observed on both sides of the staphylococcus streak. Growth could also be obtained in Difco thioglycollate broth to which was added a filtrate of *Staphylococcus* growth in peptone water.

Several experiments were performed to obtain an assay method for the growth factor. These experiments failed because (1) harvesting or washing and suspending organisms from agar plates or thioglycollate broth rendered most of

* Report on work carried out during tenure of an N.R.C. Senior Dental Research Fellowship under the direction of Dr. J.B. Macdonald at the University of Toronto, July-December 1956.

the cells non viable, and (2) the Staphylococcus growth filtrate was inactivated during the preparation of cylinder of filter strip assay methods.

References

Macdonald, J.B. et al.
The Pathogenic Components of an Experimental
Fusospirochetal Infection, J. Inf. Dis. 98: 15-
20, 1956.

Minutes of the Meeting of the Associate Committee
on Dental Research, March, 1955.

Report of Work

In order to minimise manipulations of the organisms in preparing sealed media for assay experiments of the growth factor a non agar containing medium was sought which would support growth of strain K110.

A medium was compounded corresponding to the Difco formula of thioglycollate broth with the exception of agar. To this medium was added 10 vol. per cent of filtrate of Staphylococcus aureus growth in peptone water. Two methods of inoculation were used: (1) a loopful of growth of K110 scraped from an area parallel to the staphylococcus cross streak of a blood agar plate, (2) 0.2 ml. of growth of K110 in Difco thioglycollate broth + 10 vol. per cent of staphylococcus filtrate.

I. Sensitivity to Hydrogen:

Several methods were used to reduce or partly reduce the medium before inoculation. These included:

- (a) Reducing by heat and rapid cooling of either one or both the agar free medium and the filtrate immediately before inoculation.
- (b) Storing either one or both the agar free medium and the filtrate in a hydrogen atmosphere for 24 hours before inoculation.
- (c) Combinations of (a) and (b).

Two methods of incubation were employed:

- (a) Upon inoculation the medium was immediately incubated in a Brewer jar filled with hydrogen containing 10 per cent CO₂.

- (b) Upon inoculation the medium was overlayed with a 6 to 8 cm. column of sterile paraffin oil and incubated aerobically.

Difco thioglycollate broth with the addition of 10 vol. per cent filtrate served as control medium.

Results:

The medium consistently failed to support growth when incubated in a hydrogen atmosphere. Slight growth was observed, confirmed by subculture on blood agar plates with a cross streak, in some of the tubes which had been reduced by heat and overlayed with oil incubated aerobically.

Growth failed in the medium when reduced by hydrogen for 24 hours and overlayed with oil. Growth was observed in the control medium in all cases.

Subcultures to the same agar free medium were made from the tubes showing growth and to the control medium. The same methods of reduction and incubation were employed.

Results: Slight growth occurred in some of the tubes only when reduced by heat and overlayed with oil and incubated aerobically. Growth occurred in the control medium.

Conclusion: Strain K110 is probably sensitive to free access of hydrogen in a liquid medium.

II. Addition of an Agar Surface:

Growth of K110 was observed in the agar free medium to occur mostly either as a film against the wall of the tube or around an incidental piece of agar when inoculation was performed with scrapings of growth from a blood agar plate. The presence of a large absorbent surface was tested as follows: Short thioglycollate agar slopes were submerged under agar free thioglycollate medium + 10 vol. per cent of filtrate, both reduced by heat. The medium was overlayed with oil. This medium and that without a slope was inoculated using the above described methods.

Results: More consistent growth was obtained in the tubes containing an agar slope. Growth was observed as a film on the agar slope and as a sediment at the bottom of the slope. Moderate rolling of the tube resulted in a light turbid suspension of organisms.

The influence of changes in the composition of thioglycollate medium were tested. It was found:

- (a) Changing the concentration of glucose failed to influence the number of tubes showing growth.
- (b) Beef extract and proteose peptone could be replaced by Difco heart infusion medium.
- (c) Addition of six drops of defibrinated sheep's blood resulted in an increase in the number of tubes supporting growth. In this case the occurrence of growth had to be established entirely by subculture on blood agar. A black pigmented sediment developed in the tubes giving positive subculture.

Incubation in a Nitrogen Atmosphere

In order to investigate the sensitivity of K110 for hydrogen in a fluid medium three series of subcultures were made.

Twelve tubes each containing a heart infusion slope submerged under a Difco heart infusion medium containing 0.05 per cent thioglycollate, 10 per cent filtrate and six drops of defibrinated sheep's blood (this medium was reduced by heat). Six tubes were transferred immediately upon inoculation to a Brewer jar filled with nitrogen. Six tubes were overlaid with oil and incubated aerobically.

Result: Growth occurred in three or four tubes using either incubation method.

Influence of Hydrogen and Nitrogen on Heart Infusion Media

In each of two Brewer jars were placed two tubes, one containing heart infusion medium + 0.05 per cent thioglycollate and the other the same medium + 0.05 per cent

agar. Both media were reduced by heat. A sterile gold foil electrode and agar bridge were inserted in each tube. Connection with half-cells (also placed inside the jars) was made via KCl solution. The leads from half-cell and electrode were embedded in the plasticine seal. Both jars were exhausted and refilled with hydrogen. After fifteen minutes one of the jars was again exhausted and refilled with nitrogen. The asbestos catalisars were heated for 30 minutes in each case.

Result: The potential of both media in the nitrogen atmosphere dropped only slightly over a period of six hours.

The potential of the agar free medium in the hydrogen atmosphere showed a gradual drop of over 150 mV over a period of six hours.

The potential of the agar containing medium in hydrogen did not change during the observation period.

Conclusion: Growth of K110 in fluid media is probably restricted to a limited potential range.

Method Now Used for Maintaining K110

Two series of subcultures, one of eight and one of twelve subcultures in tubes containing a heart infusion slope, were overlaid with heart infusion medium containing 0.05 per cent thioglycollate, 10 per cent filtrate of *Staphylococcus aureus* growth, + six drops of defibrinated sheep's blood. Each time the medium was reduced by storing in a nitrogen atmosphere for 24 hours. Upon inoculation the tubes were transferred to a Brewer jar. The jars were exhausted and refilled with hydrogen. After fifteen minutes the jars were again exhausted and refilled with nitrogen. The platinized asbestos catalisars were heated for 30 minutes.

Secretary's note: Dr. Madlener has also submitted a copy of his thesis entitled "Isolation and Characteristics of *Spirillum sputigenum*", submitted in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, and completed during tenure of a Graduate Dental Research Fellowship held in 1955-56.

APPENDIX Q

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

List of Grantees and Projects

<u>Project No.</u>		<u>Grantee</u>	<u>Subject</u>
DR-1	Completed	Dr. J. J. Rae, Dept. of Chemistry, Univ. of Toronto	Chemical mechanisms in dental caries.
DR-2	Completed	Dr. G. H. W. Lucas, Dept. of Pharmacology, Univ. of Toronto	A study of alveolar oxygen tension and blood oxygen content during nitrous oxide anaesthesia.
DR-3	Completed	Dr. H. K. Box, Faculty of Dentistry, Univ. of Toronto	A study of micro- organisms found in deep periodontal pockets and caries lesions as revealed by the electromicroscope.
DR-4	Completed	Dr. H. K. Box, Faculty of Dentistry, Univ. of Toronto	An investigation of packing material in the treatment of periodontal disease.
DR-5	Completed	Major R. L. Twible, Royal Cdn. Dental Corps (later O.R.F.)	To improve certain properties of the acrylic resin denture base and to effect an economy in denture production.
DR-6	Completed	Brig. E. M. Wansbrough, Royal Cdn. Dental Corps.	To study the effects of altitude accelera- tion and deceleration on oral tissues.
DR-7	Completed	Dr. J. S. Bagnall, Faculty of Dentistry, Dalhousie University	Critical survey of the literature on dental caries.
DR-8	Completed	Dr. A. D. A. Mason, Faculty of Dentistry, Univ. of Toronto	A study of the D.M.F. caries rate of the children of Brantford prior to the addition of fluorine to the water supply.

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-9 Completed	Dr. O. J. Walker, Dept. of Chemistry, Dr. H. R. MacLean, Faculty of Dentistry, Univ. of Alberta	An investigation of the effects of certain chemicals on materials in appliances used in prosthetic dentistry,
DR-10 Completed	Dr. G. M. Macdonald Queen Mary Veterans Hospital, Montreal	Facial prostheses.
DR-11 Completed	Dr. R. J. Godfrey, Faculty of Dentistry, Univ. of Toronto	An anatomical, histological and physiological study of the role of the condyle in mastication.
DR-12 Completed	Dr. A. E. R. Westman, Ont. Res. Foundation	Investigation of cements for methacrylate inlays.
DR-13 Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, Univ. of Toronto	Fluoride content of dentine, enamel and alveolar processes of teeth from Sarnia, Brantford and Stratford.
DR-14 Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, Univ. of Toronto	Fluorine analysis of water and food samples from different areas in Ontario collected and studied in relation to incidence of dental caries.
DR-15 Completed	Dr. N. F. Walker, Dept. of Zoology, Univ. of Toronto	A comparison of the patterns of eruption of the deciduous teeth in man with rate of growth of the dental arches.
DR-16 Completed	Dr. C. H. Best, Dept. of Medical Research, Univ. of Toronto	Studies <u>in vitro</u> of certain fungus-like organisms found in periodontal diseases.

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-17 Completed	Dr. R. F. Shaner, Dept. of Anatomy, Univ. of Alberta	An investigation of effects of solutions used in operative dentistry on the vital pulps of teeth.
DR-18 Completed	Dr. H. K. Box, Faculty of Dentistry, Univ. of Toronto	Experimental production of dental caries.
DR-19 Completed	Dr. E. A. Sellers, Dept. of Pharmacology, Univ. of Toronto	Tissue toxicity and efficiency of local anaesthetics for topical application and nerve block in dentistry.
DR-20 Completed	Dr. Jules Thebaud, Faculty of Dental Surgery, Univ. of Montreal	Improvement of subgingival curettage and surgical techniques used in the treatment of pyorrhea alveolaris.
DR-21 Completed	Dr. A. E. R. Westman, Ont. Res. Foundation	Investigation of repair techniques for methacrylate dentures.
DR-22 Active	Dr. C. H. Best, Dept. of Medical Research, Univ. of Toronto (later W. J. Linghorne)	Investigation of the mechanism of repair of the supporting structure of the teeth.
DR-23 Active	Dr. C. P. Leblond, Dept. of Anatomy, McGill University	Entry of phosphate, carbonate and calcium ions into teeth, as shown with the help of radioactive elements.
DR-24 Completed	Dr. C. H. M. Williams, Faculty of Dentistry, Univ. of Toronto	The effects of hard and soft diets on the supporting structures of the teeth.
DR-25 Completed	Dr. A. E. R. Westman, Ont. Res. Foundation	Investigation of methods for the use of methyl methacrylate in direct dental fillings.

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-26 Completed	Dr. R. E. Moyers, Faculty of Dentistry, Univ. of Toronto	A study of the normal and abnormal physiologic forces of the oral cavity.
DR-27 Completed	Dr. J. W. Abbiss, Faculty of Dentistry, Dr. A. G. Nutlay, Faculty of Dentistry, Dalhousie University	A histological study of tooth germ development, both intra and extra alveolar, with a survey of pathological changes in the developing tooth.
DR-28 Completed	Dr. O. J. Walker, Dept. of Chemistry, Univ. of Alberta	Investigation of methods for destroying the organic matter in teeth, bones and other materials prior to determination of fluorine content.
DR-29 Completed	Dr. M. Doreen Smith, Dept. of Food Chemistry, Univ. of Toronto	Biological absorption of fluorine.
DR-30 Completed	Dr. N. F. Walker, Dept. of Zoology Univ. of Toronto	The study of the closure of space following premature loss of deciduous teeth.
DR-31 Completed	Dr. L. B. Jaques, Dept. of Physiology, Dr. G. J. Millar, Dept. of Physiology, Univ. of Saskatchewan	An investigation of nerve block.
DR-32 Completed	Dr. A. W. Ham, Dept. of Anatomy, Univ. of Toronto	Investigation of the effect of adrenal cortical hormone imbalances on the various connective tissue structure of the growing incisor teeth of animals.
DR-33 Completed	Dr. H. A. Hunter, Faculty of Dentistry, Univ. of Toronto	A study of calcifying potential of calcium oleate on the dental pulp.

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-34 Completed	Dr. A. D'Iorio, Dept. of Physiology, Univ. of Montreal	Studies of calcium metabolism in teeth with the aid of Ca^{45} .
DR-35 Completed	Dr. J. B. Macdonald, Div. of Dental Research, Univ. of Toronto	Bacteriology of periodontal disease. I. Experimental fusospirochetal infection with mixtures of pure culture.
DR-36 Completed	Dr. G. Nikiforuk, Div. of Dental Research, Univ. of Toronto	Demineralization of hard tissues as bone and teeth by organic chelating agents.
DR-37 Completed	Dr. D. H. Jenkins, Dept. of Orthodontics, Univ. of Toronto	Further studies in the electromyography of the head, face and neck.
DR-38 Completed	Dr. K. J. Paynter, Faculty of Dentistry, Univ. of Toronto	The effects of the endocrines on the teeth and jaws. II. Further studies on the effect of thiouracil on the development of molar teeth of rats.
DR-39 Completed	Dr. A. G. Racey, Faculty of Dentistry, McGill University	Sectioning of teeth and surrounding structures and the effects of potassium deficiency and of pyridoxine deficiency on the teeth and surrounding structures of dogs.
DR-40 Completed	Dr. L. A. Funt, Faculty of Dentistry, Univ. of Toronto	Effect of tooth eruption and function on the growing of the mandible and facial complex on cats.
DR-41 Completed	Dr. D. H. Jenkins, Dept. of Orthodontics, Univ. of Toronto	A study of the dental arch form and certain of its anatomical relations in a series of clinically excellent dentitions.

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-42 Completed	Dr. G. Nikiforuk, Div. of Dental Research, Univ. of Toronto	Studies in the chemistry of the saliva. II. The reducing property of saliva towards some oxidation-reduction indicators.
DR-43 Completed	Dr. S. G. Davis, Dept. of Chemistry, Dr. H. R. MacLean, Faculty of Dentistry, Univ. of Alberta	Electro-potentials caused by dental alloys.
DR-44 Completed	Dr. A. H. Craven Faculty of Dentistry, McGill University	Facial and cranial growth patterns in the rat as revealed by vital staining with alizarine red S.
DR-45 Completed	Dr. R. E. Moyers, Faculty of Dentistry, Univ. of Toronto	Study of the incidence of malocclusion.
DR-46 Completed	Dr. D. H. Jenkins, Dept. of Orthodontics, Univ. of Toronto	Study of treated cases.
DR-47 Completed	Dr. H. A. Hunter, Faculty of Dentistry, Univ. of Toronto	The pathological characteristics of experimental fusospirochetal infections.
DR-48 Completed	Dr. G. Nikiforuk, Div. of Dental Research, Univ. of Toronto	The effect of topical application of silicones in protecting teeth against acid solutions <u>in vitro</u> and dental caries produced <u>in vitro</u> .
DR-49 Completed	Dr. A. H. Craven, Faculty of Dentistry, Univ. of Toronto	A recording device to determine postural changes of the head in the horizontal plane.
DR-50 Completed	Dr. J. B. Macdonald, Div. of Dental Research, Univ. of Toronto	The cultivation and characteristics of <u>Bacteroides nigrescens</u> .

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-51 Completed	Dr. R.L.deC.H. Saunders, Dept. of Anatomy, Dalhousie University	Study of human dental pulp blood vessels by X-ray microarterio- graphic methods.
DR-52 Active	Dr. L. F. Belanger, Dept. of Histology & Immunology, Univ. of Ottawa	Mechanism of bone and tooth formation in the light of auto- radiography.
DR-53 Completed	Dr. C. B. Weld, Dept. of Physiology, Dalhousie University	The macrophages in the pulp tissue of the rat incisor and lower jaw.
DR-54 Completed	Dr. B. N. Kropp, Faculty of Medicine, Queen's University	Development of a rapid method for grinding thin section of plastic- embedded teeth.
DR-55 Completed	Dr. M. R. Culbert, Dept. of Orthodontics, Univ. of Toronto	A cephalometric study of maxillary growth as related to statural growth.
DR-56 Completed	Dr. D. J. E. Mitchell, Dept. of Orthodontics, Univ. of Toronto	Comparative study of palatal height, width and length in ancient and modern crania.
DR-57 Completed	Dr. G. Nikiforuk, Div. of Dental Research, Univ. of Toronto	The amino acid composi- tion of enamel protein using paper chromato- graphy.
DR-58 Active	Dr. J. G. Aldous, Dept. of Pharmacology, Dalhousie University	The metabolism of procaine and related compounds.
DR-59 Active	Dr. D. H. Copp Dept. of Physiology, U. of British Columbia	Effect of phosphorus deficiency on bones and teeth.
DR-60 Completed	Dr. F. C. Fraser, Dept. of Genetics, McGill University	Pathogenesis of cortisone-induced cleft palate in mice.
DR-61 Active	Dr. A. S. V. Burgen, Dept. of Physiology, McGill University.	Secretion of protein in saliva.

<u>Project No.</u>	<u>Grantee</u>	<u>Subject</u>
DR-62 Completed	Dr. S. Kirkwood, Dept. of Biochemistry, McMaster University	Role of the salivary glands in the extra-thyroidal metabolism of the thyroid hormone.
DR-63 Completed	Dr. K. J. Paynter, Div. of Dental Research, Univ. of Toronto	A histological study of teeth and periodontal tissues of guinea pigs reared on subminimal allowances of ascorbic acid.
DR-64 Active	Dr. K. J. Paynter, Div. of Dental Research, Univ. of Toronto	Investigations into the nature of cementum.
DR-65 Active	Dr. J. G. Perreault, Faculty of Dentistry, Univ. of Montreal	An evaluation of the disturbance of the dentine formation in the rat incisor following trauma of various origins.
DR-66 Active	Dr. K. I. Melville, Dept. of Pharmacology, McGill University	Studies on the mechanism of gingival changes associated with drug administration.
DR-67 Active	Dr. K. A. McMurchy, Faculty of Dentistry, Univ. of Alberta	A study of resorption of enamel, dentin and cementum.
DR-68 Active	Dr. H. R. MacLean, Faculty of Dentistry, Univ. of Alberta	The role of epithelium in the genesis of anomalies of tooth development.

APPENDIX R

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

List of Publications

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
1	New aspects of periodontal re- search, by H. K. BOX.	Presented at National Convention of Can. Dentists, Toronto, May 29 1946 (App. C. 5th Mtg., A.C.D.R., 25 Feb. 1947)
2	Concerning the pathogenesis of periodontal disease, by H. K. BOX.	J. Periodontology 19: 11-20, 1948.
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J. J. RAE and C. T. CLEGG.	J. Dent. Res. 27: 52-53, 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J. J. RAE and C. T. CLEGG.	J. Dent. Res. 27: 54-57, 1948.
5	An improved method for processing acrylic dentures, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 14: 303-317, 1948.
6	The therapeutic properties of periodontal cement packs, by W. J. LINGHORNE and D. C. O'CONNELL.	J. Can. Dent. Assoc. 15: 199-205, 1949.
7	Phosphatase in saliva, by J. T. DENTAY and J. J. RAE.	J. Dent. Res. 28: 68-71, 1949.
8	The treatment of acute oral Vincent's infection, by W. J. LINGHORNE and D. F. STEDMAN.	J. Can. Dent. Assoc. July 1949.
9	A comparison of nine local anaesthetics, by H. S. HAMILTON, B.A. WESTFALL and J.K.W. FERGUSON.	J. Pharmacol. Expt. Ther. 94: 299-307, 1948.
10	New methods of repairing acrylic dentures without distortion, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 15: 582-590, 1949.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D. E. R. COGGLES, O. J. WALKER and H. R. MacLEAN.	J. Can. Dent. Assoc. 16: 75-82, 1950.
12	The relation between buffering capacity, viscosity and lactobacillus count of saliva, by J. J. RAE and C. T. CLEGG.	J. Dent. Res. 28: 589-593, 1949.
13	Mineralization of the growing tooth as shown by radiophosphorus autographs (17692), by L. F. BELANGER and C. P. LEBLOND.	Proc. Soc. Expt. Biol. Med. 73: 390-391, 1950.
14	Radio-autographic visualization of bone formation in the rat, by C. P. LEBLOND, C. W. WILKINSON, L. F. BELANGER and J. ROBICHON.	Am. J. Anat. 86: 2, 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH.	Can. J. Research, F, 28: 227-233, 1950.
16	Studies in the regeneration and reattachment of supporting structures of the teeth. I. Soft tissue reattachment, by W. J. LINGHORNE and D. C. O'CONNELL.	J. Dent. Res. 29: 419-428, 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH.	J. Can. Dent. Assoc. Jan., 1951.
18	Uptake of radioactive calcium by the dental tissues of the young rat, by A. D'IORIO and J. P. LUSSIER.	Rev. Canadienne de Biol.
19	Relining acrylic dentures without distortion, by D. R. CHRISTIE.	J. Can. Dent. Assoc.
20	Acrylic fillings - general comments and some experimental data, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 17: 427-435, 1951.
21	Ammonia production in saliva, by R. M. BALLANTYNE, C. T. CLEGG, J. J. RAE and F. H. LAWFORD.	J. Dent. Res. 30: 385-387, 1951.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
22	Studies in the regeneration and reattachment of supporting structures of the teeth. II. Regeneration of alveolar process, by W. J. LINGHORNE and D. C. O'CONNELL.	J. Dent. Res. 30: 604-614, 1951.
23	Radioactive isotopes in dental research, by J. P. LUSSIER and A. D'IORIO.	The Brit. Dent. Annual, 1952.
24	Determination of fluoride in water by the aluminum-hematoxylin method, by MARGARET J. PRICE and O. J. WALKER.	Anal. Chem. 24: 1953, 1952.
25	Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat, by L. F. BELANGER.	Science 118: 520-521, 1954.
26	The motile non-sporulating anaerobic rods of the oral cavity, by J. B. MACDONALD.	University of Toronto Press, Toronto, 1953.
27	Motility in a species of non-flagellated bacteria (20677), by J. B. MACDONALD, M. L. KNOLL and R. M. SUTTON.	Proc. Soc. Expt. Biol. & Med. 84: 459-462, 1953.
28	The periodontium and salivary glands in the alarm reaction, by G. SHKLAR and I. GLICKMAN.	J. Dent. Res. 32: 773-778, 1953.
29	Autoradiographic visualization of S^{35} incorporation and turnover by the mucous glands of the gastrointestinal tract and other soft tissues of rat and hamster, by L. F. BELANGER.	Anat. Rec. 118: 775-772, 1954.
30	Autoradiographic visualization of the entry and transit of S^{35} in cartilage, bone, and dentine of young rats and the effect of hyaluronidase <u>in vitro</u> , by L. F. BELANGER.	Can. J. Biochem. & Physiol. 32: 161-169, 1954.

Paper No.	Title and Author	Reference
31	Autoradiographic visualization with Ca^{45} of normal growth of the incisor of pigs and the effect of fluorine feeding, by L. F. BELANGER, W. E. LOTZ, W. J. VISEK and C. L. COMAR.	Anat. Rec. 119: 53-70, 1954.
32	Fluorine balance studies of four infants, by MARY P. HAM and M. DOREEN SMITH.	J. Nutrition 53: 215-223, 1954.
33	Fluorine balance studies of three women, by MARY P. HAM and M. DOREEN SMITH.	J. Nutrition 53: 225-232, 1954.
34	The effect of propylthiouracil on the development of molar teeth, by K. J. PAYNTER.	J. Dent. Res. 33: 364-376, 1954.
35	Autoradiographic detection of radiosulfate incorporation by the growing enamel of rats and hamsters, by L. F. BELANGER.	J. Dent. Res. 34: 20-27, 1955.
36	Autoradiographic visualization of Ca^{45} intake by normal and pathological cartilage <u>in vitro</u> , by L. F. BELANGER.	Proc. Soc. Expt. Biol. & Med. 88: 150-152, 1955.
37	Studies in the reattachment and regeneration of the supporting structures of the teeth. III. Regeneration in epithelized pockets, by W. J. LINGHORNE and D. C. O'CONNELL.	J. Dent. Res. 34: 164-177, 1955.
38	Ammonia production in saliva. I., by J. J. RAE, H. M. SHELILT and C. T. CLEGG.	J. Dent. Res. 34: 886-888, 1955.
39	Growth in width of the head of the Macaca rhesus monkey as revealed by vital staining, by A. H. CRAVEN.	Am. J. Orthodontics 42: 341-362, 1956.
40	Preparing single or serial section of calcified bones and teeth, by B. N. KROPP.	Stain Tech. 31: 253-261, 1956.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
41	The effects of single injections of soluble components of "fusospirochetel" materials in experimental animals, by H. A. HUNTER and J. B. MACDONALD.	J. Dent. Res. 35: 4-8, 1956.
42	Mixed infections of the oral cavity, by J. B. MACDONALD.	J. Norwegian Dent. Assoc. April 1956
43	The pathogenic components of an experimental fusospirochetel infection, by J. B. MACDONALD, R. M. SUTTON, M. L. KNOLL, E. M. MADLENER and R. M. GRAINGER.	J. Infectious Dis. 98: 15-20, 1956.
44	The production of fusospirochetel infections in guinea pigs with recombined pur cultures, by J. B. MACDONALD, R. M. SUTTON and M. L. KNOLL.	J. Infectious Dis. 95: 275-284, 1954.
4.	Dr. J. S. Fagnall, Faculty of Dentistry, Dalhousie University, Halifax, N. S.	31 March 1957
5.	Dr. C. D. Husband, Suite 110, Metro Block, Red Deer, Alberta	31 March 1957
6.	Dr. Jean-Paul Lussier, Faculty of Dentistry, University of Montreal, Montreal, P. Q.	31 March 1957
7.	Dr. R. J. O'Neill, 2525 Pine Street, Vancouver, B. C.	31 March 1957
8.	Dr. A. G. Hacey, Faculty of Dentistry, McGill University, Montreal, P. Q.	31 March 1957
9.	Dr. C. H. M. Williams, Associate Professor of Periodontology, Faculty of Dentistry, University of Toronto, Toronto 5, Ontario	31 March 1957

APPENDIX S

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment

	<u>Term to expire</u>
★ 1. Dr. J. B. Macdonald, <u>Chairman</u> , Director, Forsyth Dental Infirmary for Children, 140 The Fenway, Boston 15, Mass.	31 March 1957
2. Dr. E. W. R. Steacie, <u>ex officio</u> President, National Research Council, Ottawa 2, Ontario	- - -
3. Dr. D. L. Thomson, <u>ex officio</u> Vice-Principal, McGill University, Montreal, P. Q.	- - -

Representing the Dental Profession

4. Dr. J. S. Bagnall, Faculty of Dentistry, Dalhousie University, Halifax, N. S.	31 March 1958
5. Dr. C. D. Husband, Suite 110, Metro Block, Red Deer, Alberta	31 March 1959
6. Dr. Jean-Paul Lussier, Faculty of Dentistry, University of Montreal, Montreal, P. Q.	31 March 1957
7. Dr. R. J. O'Neil, 2525 Pine Street, Vancouver, B. C.	31 March 1959
★ 8. Dr. A. G. Racey, Faculty of Dentistry, McGill University, Montreal, P. Q.	31 March 1959
9. Dr. C. H. M. Williams, Associate Professor of Periodontology, Faculty of Dentistry, University of Toronto, Toronto 5, Ontario	31 March 1957

★ Member of Executive.

Term to expireRepresenting the Royal Canadian Dental Corps,
Department of National Defence

- * 10. Brig. E. M. Wansbrough, ex officio
Director General of Dental Service,
Royal Canadian Dental Corps,
Dept. of National Defence,
Ottawa, Ontario

Representing the Division of Dental Health,
Department of National Health and Welfare

11. Dr. H. K. Brown, ex officio
Chief, Dental Health Division,
Dept. of National Health and Welfare,
Ottawa, Ontario

Representing Dental Research,
Department of Veterans Affairs

12. Dr. L. A. Kilburn, ex officio
Director of Dental Research,
Dept. of Veterans Affairs,
Ottawa, Ontario

Representing the Basic and Medical Sciences

- | | |
|--|---------------|
| 13. Dr. J. G. Aldous,
Dept. of Pharmacology,
Dalhousie University,
Halifax, N. S. | 31 March 1959 |
| 14. Dr. H. J. Barrie,
Dept. of Pathology,
University of Toronto,
Toronto 5, Ontario | 31 March 1957 |
| 15. Dr. J. M. R. Beveridge,
Dept. of Biochemistry,
Queen's University,
Kingston, Ontario | 31 March 1959 |
| 16. Dr. A. C. Burton,
Dept. of Biophysics,
University of Western Ontario,
London, Ontario | 31 March 1957 |

* Member of Executive.

Term to expire

17. Dr. D. H. Copp,
Dept. of Physiology,
University of British Columbia,
Vancouver, B. C.

31 March 1959

★ 18. Dr. A. W. Ham,
Dept. of Anatomy,
University of Toronto,
Toronto 5, Ontario

31 March 1957

19. Dr. Edouard Pagé,
Dept. of Biology,
University of Montreal,
Montreal, P. Q.

31 March 1958

★ 20. Dr. J. B. Marshall, Secretary
National Research Council,
Ottawa, Ontario

- - -

★ Member of Executive

21. Alberta - Dr. W. B. Hamilton

22. Dalhousie - Dr. J. D. McLean

23. McGill - Dr. D. Prescott Mowry

24. Toronto - Dr. R. G. Ellis

25. Board Room copy

26. Library

27-35 Reserve

INITIAL DISTRIBUTION

(i) To members of the

Associate Committee on Dental Research

- | | |
|---|--|
| 1. Dr. J. B. Macdonald, <u>Chairman</u> | 11. Dr. L. A. Kilburn |
| 2. Dr. J. G. Aldous | 12. Dr. J-P. Lussier |
| 3. Dr. J. S. Bagnall | 13. Dr. R. J. O'Neil |
| 4. Dr. H. J. Barrie | 14. Dr. E. Pagé |
| 5. Dr. J. M. R. Beveridge | 15. Dr. A. G. Racey |
| 6. Dr. H. K. Brown | 16. Dr. E. W. R. Steacie |
| 7. Dr. A. C. Burton | 17. Dr. D. L. Thomson |
| 8. Dr. D. H. Copp | 18. Brig. E. M. Wansbrough |
| 9. Dr. A. W. Ham | 19. Dr. C. H. M. Williams |
| 10. Dr. C. D. Husband | 20. Dr. J. B. Marshall, <u>Secretary</u> |

(ii) To other persons:-

21-24 Deans of Dental Schools (who are not members of the
Committee)

- | | |
|---------------|-------------------------|
| 21. Alberta | - Dr. W. S. Hamilton |
| 22. Dalhousie | - Dr. J. D. McLean |
| 23. McGill | - Dr. D. Prescott Mowry |
| 24. Toronto | - Dr. R. G. Ellis |

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY - FOURTH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

MONTREAL

MARCH 3 - 4, 1958

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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PART I

Initial Distribution open session of the Committee convened at 9:30 a.m. in the Amphitheatre on the 6th floor of the Montreal General Hospital, 1625 Pine Avenue, Montreal. This session was open to members of the student body and faculty of the University of Montreal and of McGill University, and to members of the profession.

The members of the Committee and its guests were welcomed by Dean J. E. McCutcheon of McGill University Dental School; Dr. J.-P. Lussier then took the chair.

During the morning session, reports were presented by

- Dr. G. P. Leblond - "Stability of dentin"
- Dr. L.-F. Bélanger - "Intimate mechanism of mineralization"
- Dr. D. H. Copp - "Phosphorus deficiency - effects on bones and teeth"
- Dr. J. G. Alenus - "Metabolism of proline and related compounds"
- Dr. K. I. Melville - "Gingival changes associated with drug administration"
- Dr. K. J. Paynter - "Investigation into the nature of cementum"

The meeting adjourned at 1:15 p.m. for luncheon in the cafeteria of the Montreal General Hospital and recommenced at 2:00 p.m. in the Amphitheatre. Dr. J. E. Macdonald took the chair.

NATIONAL RESEARCH COUNCIL

Proceedings of the Twenty-fourth Meeting of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Montreal

March 3 - 4, 1958

PART I

The first open session of the Committee convened at 9:30 a.m. in the Amphitheatre on the 6th Floor of the Montreal General Hospital, 1625 Pine Avenue, Montreal. This session was open to members of the student body and faculty of the University of Montreal and of McGill University, and to members of the profession.

The members of the Committee and its guests were welcomed by Dean J. S. McCutcheon of McGill University Dental School; Dr. J.-P. Lussier then took the chair.

During the morning session, reports were presented by

Dr. C. P. Leblond - "Stability of dentin"

Dr. L.-F. Belanger - "Intimate mechanism of mineralization"

Dr. D. H. Copp - "Phosphorus deficiency - effects on bones and teeth"

Dr. J. G. Aldous - "Metabolism of procaine and related compounds"

Dr. K. I. Melville - "Gingival changes associated with drug administration"

Dr. K. J. Paynter - "Investigation into the nature of cementum"

The meeting adjourned at 1:15 p.m. for luncheon in the cafeteria of the Montreal General Hospital and reconvened at 2:00 p.m. in the Amphitheatre. Dr. J. B. Macdonald took the chair.

During the afternoon session, reports were presented by

- Dr. A. M. Hunt - "Periodontal tissues of normal and scorbutic guinea pigs"
- Dr. M. Weiss - "Synthesis and excretion of protein in saliva"
- Dr. G. Perreault and Dr. G. Pelletier - "Effects of acute and chronic trauma on the pulp and growing dentin in the incisors of albino rats"
- Dr. K. A. McMurchy - "Resorption of enamel, dentin and cementum" (presented by Dr. C. Castaldi)
- Dr. K. A. McMurchy and Dr. R. D. Haryett - "Study of traumatic (surgical) tooth movement" (presented by Dr. C. Castaldi)

Following presentation of the reports, the Chairman introduced Dr. Copp.

Dr. Copp, as Chairman of the Associate Committee on Dental Research, thanked the students and faculty members for attending the meeting, and the grantees and fellows for the very interesting papers presented on work supported by the Committee. He said that the field of dental research had only begun to develop in Canada and he looked forward to additional work being undertaken by those still in Dental Schools.

For the benefit of those who were unfamiliar with the activities of the Committee, Dr. Copp gave a brief outline of the historical background and enlarged on the methods by which the National Research Council, through its Associate Committee on Dental Research, endeavours to promote dental research in Canada.

The Committee's main concern is to develop an interest among dental graduates in research as a career and to this end has a three-part programme:

- 1) Dental Research Assistantships which are awarded competitively to undergraduate dental students to enable them to spend the summer in research laboratories;
- 2) Dental Research Fellowships available on a competitive basis to graduates of Canadian Dental Schools who want training in the investigative, rather than the clinical area;

PART 3) Dental Research Associateships, established in principle for senior people with training in research who will be paid on the same scale as research officers of the National Research Council's own laboratories but will be honorary members of the staff of, and carry out their work in, Canadian universities.

In addition, the Committee awards grants in aid of research in support of projects undertaken by members of the staffs of universities across the country.

In closing, Dr. Copp invited all those present to attend the evening lecture to be given at the University of Montreal by Dr. Reidar Soggnaes.

The session adjourned at 5:15 p.m.

A reception was held for the Committee and its guests by the University of Montreal at 6:00 p.m. at the "Salon des professeurs" of the Students' Social Centre, 2222 Maplewood Avenue. This was followed by dinner at 7:00 p.m. in the main dining room of the Social Centre.

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PART II

A second open session was held at 8:30 p.m., March 3rd, in Room H'404 of the University of Montreal.

Dr. Lussier took the chair and, in French, briefly outlined the history and activities of the Associate Committee on Dental Research. He then called upon Dr. Copp to introduce the guest speaker of the evening, Dr. Reidar Soggnaes, Associate Dean, and Director of the Department of Dental Research, of the Harvard School of Dental Medicine.

Dr. Soggnaes gave a most interesting and stimulating lecture on "Concepts of construction and destruction of bones and teeth" which was warmly received by all present. He was thanked by Dr. R. F. Farquharson, Vice-President (Medical) of the National Research Council.

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PART III

The business meeting of the Committee convened at 9:30 a.m., March 4th, in the Council Hall of the Faculty of Medicine of the University of Montreal.

1. Attendance

Dr. D. H. Copp, Chairman
Dr. J. G. Aldous
Dr. H. J. Barrie
Dr. J. M. R. Beveridge
Dr. C. Castaldi
Dr. R. F. Farquharson
Dr. J. P. Lussier
Dr. J. B. Macdonald
Dr. J. B. Marshall
Dr. R. J. O'Neil
Dr. E. Pagé
Dr. K. J. Paynter
Dr. G. D. Scott
Brig. E. M. Wansbrough
Dr. C. H. M. Williams
Miss D. J. Wright, Secretary

Regrets were received from

Dr. H. K. Brown
Dr. C. D. Husband
Dr. L. A. Kilburn
Dr. D. L. Thomson

2. Chairman's Remarks

In opening the business session of the meeting, the Chairman expressed the pleasure of the Committee in having Dr. Farquharson present, and also welcomed the new members of the Committee, Dr. G. David Scott of the University of Toronto, and Dr. C. Castaldi of the University of Alberta.

3. Minutes of the Twenty-third Meeting

It was MOVED by Dr. Aldous and SECONDED by Brig. Wansbrough

THAT the Minutes of the Twenty-third Meeting be taken as read, and approved.

CARRIED

4. Business Arising from the Minutes

(i) Disposition of teeth collected in Brantford study
(23rd Mtg., Min. 4(i))

The Chairman read a letter from Dr. H. K. Brown to the effect that the stock of 121 permanent and 222 deciduous teeth obtained from the sample of children in the Brantford study had been requested by Dr. Nikiforuk of the University of Toronto and had subsequently been passed on to him. Dr. Brown further advised the Committee that specimens freshly extracted from the same sample of native continuous resident children were being collected and preserved in 10% unbuffered aqueous solution of formalin. This collection of wet preserved specimens was begun in February 1958 and very few have so far been obtained, but Dr. Brown indicated that he would be glad to make them available, at the discretion of the Committee, to any interested laboratory.

The Committee was glad to know that specimens were still being preserved, and it was agreed that the method of storage must be carefully considered. Dr. Beveridge felt that formalin might not be the best preservative; Dr. Pagé thought the solution should be buffered; Dr. Copp suggested that dry storage in a frozen state or at very low temperatures might be advantageous. After brief discussion, it was agreed that since Dr. Nikiforuk has been using this type of material, Dr. Paynter and Dr. Williams should ask Dr. Nikiforuk for his recommendations as to the best methods of preserving the specimens.

(ii) Publication of research papers in the Journal of the Canadian Dental Association (23rd Mtg., Min. 4(iv))

The Chairman reported that the resolution adopted by the Committee at its 23rd Meeting had been forwarded to the Canadian Dental Association. He then read the reply which indicated that the Association would be willing to publish scientific papers in its Journal, although it was pointed out that a suitable balance between papers on basic research and on clinical aspects of dentistry must be maintained. Following receipt of this letter, a memorandum had been sent to all grantees of the Committee advising them that the C.D.A. Journal would consider publication of scientific papers submitted to its Editor; as far as could be determined, none of the grantees had submitted papers to the Journal. Dr. Lussier pointed out that one deterrent to publishing in it was that it was not abstracted by "Chemical Abstracts" and papers appearing in it were therefore not made known to workers in the dental research field.

During the course of the discussion which followed, the Chairman pointed out that it was the custom to have the Secretary send a brief report on the annual meeting of the Committee to the Editor of the C.D.A. Journal for publication. It was suggested that, this year, brief summaries of the reports presented by the grantees and fellows at the open session on March 3rd might also be sent to the Editor. In this way the members of the profession might be made more aware of work being done in dental research in Canada. Dr. Pagé felt that the publication of a series of abstracts might be more effective than the publication of the occasional complete paper.

It was MOVED by Dr. Aldous and SECONDED by Dr. Macdonald

THAT the Secretary's report to the Canadian Dental Association be expanded to include short abstracts, prepared by the grantees, of the papers presented at the meeting.

CARRIED

It was agreed that letters should be sent to the grantees who had submitted summaries of their year's work, telling them of the Committee's plan and asking them if they would be agreeable to the publication of their abstract in the Journal and, if so, whether they would care to revise it. It was also suggested by Dr. Pagé that the abstracts submitted to the C.D.A. Journal should be accompanied by a covering letter to be written by the Chairman.

Dr. O'Neil expressed the opinion that the Committee should publish an annual report comprised of papers on the work it supports, which could be distributed to the dental schools. It was felt that this might be done if a Canadian Journal of Dental Research were ever established under the auspices of the National Research Council, but that the Committee could not undertake to do this at the present time.

(iii) Release of encumbrances (23rd Mtg., Min. 8 and 9)

The Chairman reported that, following receipt of further information after the last meeting, the funds encumbered for Dr. Linghorne's project (\$1,500) and Dr. MacLean's project (\$2,252) had been released by the Executive. (It was noted that Dr. MacLean had subsequently relinquished his grant because his assistant had moved to Montreal before the project could get under way.) It was further reported that the encumbrance of \$3,000 had been released to Dr. Burgen to enable him to employ Dr. Weiss on his project, following the termination of the latter's Fellowship.

It was MOVED by Dr. Macdonald and SECONDED by Brig. Wansbrough

THAT the action of the Executive be approved.

CARRIED

(iv) Undergraduate Dental Research Assistantships
(23rd Mtg., Min. 14)

The Chairman advised the Committee that the recommendation that a limited number of dental undergraduates be employed by grantees during the summer had been acted upon. A brief report of the plan's operation in 1957 appears in APPENDIX "A".

There was some discussion of the value of the programme. Dr. Aldous felt that the students gained useful experience and that some of the techniques they learned would be of use to them, but he doubted that the plan would go very far toward developing research investigators. Dr. Copp said that while it was impossible to train a research worker in one summer, the programme might stimulate an interest in research on the part of at least a few of the undergraduates employed in grantees' laboratories; he felt that if this were achieved, in even a few cases, and the students later went into research, the programme could be regarded as worthwhile. Meanwhile, it was up to the Committee to decide whether this possibility justified the continuance of the programme.

It was MOVED by Brig. Wansbrough and SECONDED by Dr. Castaldi

THAT the programme be continued.

CARRIED

The operation of the programme was then considered at some length. Dr. Aldous suggested that students be employed on research projects at as early a stage of their academic career as possible; for this reason he felt that Assistantships should be awarded to first year students, even though students with more academic training might be more helpful to the project director.

The desirability of continuity was mentioned. Dr. Paynter suggested that Assistantships be offered to a number of good first year students, that these same students be supported in the same laboratories for the three consecutive summers of their dental training, and that the process then be repeated. Dr. Macdonald felt that the competition should not be limited in this way, although students re-applying

after having had one Assistantship should be encouraged, if their standing warranted it, by the award of a further Assistantship.

Dr. Beveridge said that, in his opinion, the Committee should not concern itself in future years with the employment of undergraduates in grantees' laboratories. He felt that the matter could best be handled by the grantees themselves, since they knew the students at their own universities and they know how much money they have available for assistants. The Chairman said that the grantees could not have been expected to act on the Committee's recommendation last year because the plan had been evolved too late for them to apply for the necessary funds; he also expressed the opinion that if the Committee operated the programme it received more publicity and by putting the jobs on a competitive basis the award of an Assistantship carried with it a certain degree of prestige. He pointed out that grantees of the Committee could at any time have employed dental undergraduates on their projects during the summer but they had not done so until the Committee took action in the matter. Dr. Beveridge said that interest in the programme had now been aroused among the students and if the number of available positions were announced at the beginning of the year the students would make their own arrangements with the grantees.

Dr. Marshall felt that Dr. Beveridge's suggestion should be seriously considered. He pointed out that the Assistantships should not be regarded as Scholarships in any sense of the word because the Council does not offer undergraduate scholarships. He suggested that the Committee might, however, act as a clearing house to provide students and grantees with information as to the availability of employment opportunities, and students qualified to fill them.

Dr. Macdonald suggested that one Assistantship be made available in each Dental School, the recipient to be chosen by the School on the basis of academic standing, etc. It was pointed out, however, that all Schools might not always have a good candidate.

It was finally agreed that after the programme has been in operation for a few years there will be no advantage in the Committee being directly involved. If the students like the programme, candidates will apply through the local grantees.

It was MOVED by Dr. Paynter and SECONDED by Dr. Williams

(v) Dental Research Associateships (23rd Mtd., Min. 15)

The Chairman asked Dr. Farquharson to speak to this.

Dr. Farquharson said that although the Council does not support any intramural research in the medical sciences, this does not mean that Council has no interest in that field. One of the recently established methods of supporting medical research is through Medical Research Associateships, eight of which are now being held in Canadian universities. Dr. Farquharson outlined the qualifications required by a Medical Research Associate and the regulations governing his appointment. A copy of the regulations appears in APPENDIX "B".

The Chairman advised the Committee that, after considering its recommendation last year, Council had approved in principle the awarding of Dental Research Associateships on the same basis, should suitable candidates for such an appointment present themselves.

Dr. Macdonald pointed out that a candidate for such a post need not necessarily hold a dental degree, as long as he was working in dental research, but he must be able not only to do research himself, but to guide others in research. Dr. Aldous added that the Committee would have to be very sure of a candidate's qualifications before appointing him as a Dental Research Associate; the necessity of providing continuing support for such an appointee would mean in effect that he would have first claim to funds available to the Committee for grants-in-aid, although his application for funds would still be subjected to critical review.

The Committee expressed its appreciation of Council's approval, in principle, of the establishment of Dental Research Associateships but agreed that no action could be taken to make such an appointment yet.

5. Action by Executive Since Last Meeting

The Chairman reported that an application for a grant of \$1,095 had been received from Dr. K. A. McMurchy and Dr. R. D. Haryett of the University of Alberta in support of a "Study of traumatic (surgical) tooth movement". In view of the fact that Dr. MacLean had relinquished his grant, funds were available to the Committee and, following circulation of the application to the members of the Executive, an award in the amount of \$1,095 was made.

It was MOVED by Dr. Paynter and SECONDED by Dr. Williams

THAT the action of the Executive be approved.

CARRIED

6. Applications for Fellowships for 1958-59

Six applications for Graduate Dental Research Fellowships were considered. The Chairman reported that the Executive had very carefully scrutinized these applications at a meeting the previous evening which had also been attended by several other members of the Committee at large.

The following action was taken, on motions moved and seconded by the members indicated:

<u>Name</u>	<u>To Work At</u>	<u>Stipend</u>
Blackmore, R. V. D.D.S.	University of Rochester School of Medicine and Dentistry, Dept. of Bacteriology	APPROVED \$4,000 (Macdonald - Beveridge)
Dale, J. G. B.A., D.D.S.('58)	Harvard School of Dental Medicine, and Forsyth Dental Infirmary, Boston	APPROVED \$3,600 (Paynter - Williams)
Friesen, J. D.D.S.	Harvard School of Dental Medicine and Forsyth Dental Infirmary, Boston	NOT APPROVED
Kelln, E. E. B.Sc., D.D.S.	University of Minnesota	NOT APPROVED
Socransky, S. S. D.D.S.	Harvard School of Dental Medicine and Forsyth Dental Infirmary, Boston	APPROVED \$3,600 (Aldous - Beveridge)
Weiss, M. B.Sc., D.D.S., M.Sc.	Dept. of Histology, McGill University	NOT APPROVED

When the Committee reviewed Dr. Friesen's application, Dr. Macdonald pointed out that there was some doubt as to whether Dr. Friesen would be accepted for the three-year programme at Harvard even if he were to obtain a Fellowship, and he had indicated that he did not wish to take advantage of the opportunity offered him to enter into a one-year programme there. It was also felt that Dr. Friesen's primary interest was in teaching and this conflicted with the purpose of the Fellowships offered by the Committee, which was not to train teachers but to promote research. It was suggested that

since Dr. Friesen had an excellent academic record and hoped eventually to join the staff of the new Dental School at the University of Manitoba, he should take advantage of the opportunity to work for one year with Dr. Macdonald at Harvard; to enable him to do so, he should seek support from the Research Committee of the Canadian Dental Association and possibly from Dean Neilson of the University of Manitoba. It was agreed that the Chairman should write to Dean Neilson informing him of the Committee's views.

In Dr. Weiss' case, the Committee felt that while it could not recommend a Fellowship, some provision should be made for his continued support under Dr. Burgen's grant until the end of June, 1958. It was therefore MOVED by Dr. Macdonald and SECONDED by Dr. Beveridge

THAT the amount of \$1,000 be encumbered to permit Dr. Burgen to continue Dr. Weiss' present term of employment until the end of June 1958, if necessary.

CARRIED

7. Applications for Grants, 1958-59

The Chairman advised the Committee that the Council had recently undertaken to award a certain number of grants on a term basis, and asked Dr. Marshall to expand on this. Dr. Marshall said that Term Grants might be awarded to established investigators who have an established research programme. These grants are made on a three-year term basis, the same amount being provided each year without the necessity of re-applying during the term. The recipient of such a grant is able to use the funds provided as he sees fit, in carrying out the project outlined in his application; projects supported in this way are reviewed every three years. The Council hopes that this type of grant will enable established investigators to plan ahead, with the assurance of funds for longer periods than was formerly possible when applications were required each year from all grantees.

Mention was also made of Non-recurring Equipment Grants for which application could be made to enable an investigator to purchase a large item of equipment necessary to his research. Formerly such grants had been paid, on the recommendation of the Committee concerned as approved by Council, from the Council's general funds. Beginning with the current year, however, grants for non-recurring equipment would be a charge against the allotments of the Committees concerned.

It was noted that several applicants had included in their grant applications an amount for payment of a Dental

Undergraduate Research Assistant during the summer months. This amount was deducted from the grant applications, to be added after consideration of Assistantship applications, to the funds awarded to grantees in whose laboratories Assistantships were to be held.

All applications for grants were then carefully reviewed by the Committee.

When Dr. Linghorne's application was considered the Chairman read to the meeting a letter from Dr. Best to Dr. Farquharson in support of Dr. Linghorne's application, and eight letters addressed either to Dr. Best or Dr. Linghorne in the last three years by American periodontists who had admired Dr. Linghorne's work. It was the opinion of the Committee that the work on Dr. Linghorne's project D.A. 22 has developed in two stages: the early years during which the grantee explored his technique and produced some useful findings, and the past three years which seemed to have been unproductive. Dr. Barrie felt that more work seemed to have been done on the project during the past year than was apparent during the preceding years; he reported that Dr. Linghorne has discontinued his work on the epithelium and is now studying alveolar bone. Dr. Barrie also drew the attention of the Committee to Dr. Linghorne's apparent success in using his periodontal pack in healing post-operative osteomyelitis. Some members felt, however, that this pack had no advantage over the more classical methods of dealing with this clinical problem.

It was eventually MOVED by Brig. Wansbrough and SECONDED by Dr. Barrie

THAT a grant of \$1,950 be awarded to Dr. Linghorne.

CARRIED

An amendment was later MOVED by Dr. Scott and SECONDED by Dr. O'Neil

THAT a grant of \$1,500 be awarded to Dr. Linghorne.

CARRIED

It was noted that Rhesus monkeys are being used by Drs. McMurchy and Haryett in their experiments on traumatic tooth movement (D.A. 69). Dr. Beveridge pointed out that this type of monkey is a carrier of the B. virus and proper caution should, therefore, be exercised by the experimenters in handling the animals. Dr. Marshall reminded the Committee that the Council assumes no liability in cases of accident or

disease contracted during experiments carried out under its grants.

Dr. Macdonald told the Committee that he had been very impressed with the work so far accomplished on project D.A. 66 as reported by Dr. Melville at the open session; he noted that it had been shown that there were biochemical differences in the tissues of animals provided with Dilantin. Dr. O'Neil suggested that Dr. Melville's work bore some relationship to gingival hyperplasia in humans. Dr. Barrie felt that the clinical and microscopic characteristics observed on the basis of the animal experiments were quite different from those of hyperplasia in humans and suggested that Dr. Melville might find it useful to consult the Department of Histology at McGill in this connection.

The following action was taken on motions moved and seconded by the members indicated:

<u>Name</u>	<u>Title and Amount Requested</u>	<u>Recommended</u>
Belanger, L. F. Univ. of Ottawa	Autoradiographic and histochemical observations on bones and teeth in lathyrisms \$4,525	\$4,500 Term Grant for 3 years (Aldous-Williams)
Burgen, A. S. V. McGill Univ.	1. Synthesis and secretion of protein in saliva 2. Secretion of bicarbonate ions in saliva \$4,050	\$4,050 (Paynter-Beveridge)
Copp, D. H. Univ. of B. C.	Phosphorus deficiency: effects on bones and teeth \$6,305	\$5,300 Term Grant for 3 years (Beveridge-Wansbrough)
Leblond, C. P. McGill Univ.	Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements \$3,850	\$3,850 (Macdonald-Wansbrough)
Linghorne, W. J. Univ. of Toronto	An investigation of the mechanisms of maintenance and of repair of the periodontium \$1,950	\$1,500 (Scott-O'Neil)

<u>Name</u>	<u>Title and Amount Requested</u>	<u>Recommended</u>
Lussier, J.-P. Univ. of Montreal	Hormonal factors and their relationships to citrate metabolism in bones and teeth \$5,300	\$5,300 (Scott-Macdonald)
Madlener, E. M. Univ. of Toronto	The characterization of an experimental "fusospirochetal" lesion in guinea pigs \$3,500	\$3,500 (Macdonald-Paynter)
McMurchy, K. A. Univ. of Alberta	A study of resorption of enamel dentin and cementum \$4,000	\$3,000 (Castaldi-Paynter)
McMurchy, K. A. Haryett, R. D. Univ. of Alberta	Study of traumatic (surgical) tooth movement \$2,157.50	\$1,160 (Paynter-Beveridge)
Melville, K. I. McGill Univ.	Studies on the mechanism of gingival changes associated with drug administration \$7,615	\$4,000 (Macdonald-Williams)
Nikiforuk, G. Univ. of Toronto	Micro balance \$676.50	\$676.50 Non-recurring Equipment Grant (Wansbrough-Aldous)
Paynter, K. J. Univ. of Toronto	Investigations into the nature of cementum \$2,000	\$2,000 (Williams-Wansbrough)
Paynter, K. J. Hunt, A. M. Univ. of Toronto	The relation of nutrition to morphology and caries susceptibility in the teeth of rats \$3,900	\$2,700 (Williams-Wansbrough)
Paynter, K. J. Univ. of Toronto	Microtome, and accessories \$1,850	WITHDRAWN
Perreault, G. Univ. of Montreal	Effects of acute and chronic trauma on the pulp and growing dentin in the incisors of albino rats \$640	\$640 (Lussier-Castaldi)

<u>Name</u>	<u>Title and Amount Requested</u>	<u>Recommended</u>
Szerb, J. C. Dalhousie Univ.	Pharmacological analysis of the autonomic innervation of the isolated guinea pig ileum \$1,220	NOT APPROVED
Tegart, R. L. Univ. of Alberta	Study of inflammatory response to sterile glass rods and cannulae in connective tissue \$530	\$530 (Castaldi-O'Neil)

In all, 17 applications totalling \$54,069 were considered; approval of the following was recommended:

12 Annual Grants totalling	\$32,230.00
2 Term Grants totalling	9,800.00
1 Non-recurring Equipment Grant	676.50

8. Applications for Summer Dental Research Assistantships

The Committee now turned its attention to applications for Summer Dental Research Assistantships. Of the 23 applications received, 6 were from the University of Toronto, 5 from the University of Montreal, 3 from the University of Alberta, 3 from Dalhousie University and 6 from McGill University, 3 of the applicants were completing 1st year of the Dental course, 14 were completing 2nd year and 6 were completing 3rd year.

In view of the funds available to the Committee it was MOVED by Dr. Aldous and SECONDED by Dr. Pagé

THAT five Assistantships be offered for the summer of 1958.

CARRIED

Dr. Williams reported that as requested by the Committee, he and Dr. Beveridge had reviewed the summaries of the individual applications, had first selected those of highest merit and then from the point of view of geographical distribution nine names were submitted to the Committee for consideration. The Committee agreed that the following five applicants be offered employment for four months during the summer of 1958 in the laboratories of grantees of the Committee as indicated.

<u>Name</u>	<u>To Work With</u>	<u>Salary</u>
Andreas, F. 2nd yr. student, Univ. of Toronto	Dr. K. J. Paynter	\$285 per month
Carr, E. D. 2nd yr. student, Univ. of Alberta	Dr. K. A. McMurchy	\$285 per month
Demircioglu, A. 1st yr. student, Univ. of Montreal	Dr. J.-P. Lussier	\$265 per month
Fletcher, R. G. 1st yr. student, McGill Univ.	Dr. C. P. Leblond	\$265 per month
Paszti, M. 2nd yr. student, Univ. of Toronto	Dr. E. M. Madlener	\$285 per month

An amount totalling \$5,540 was therefore encumbered, to be added to the grants of the investigators noted above, if the Assistantships are accepted as offered.

9. Finances

(i) Financial Status of Committee as at February 28, 1958

The Chairman reported that the Committee now had at its disposal only sufficient funds remaining unexpended from its 1957-58 allotment to cover the cost of the 24th meeting.

Dr. Aldous reported that some \$1,500 of his 1957-58 grant remained unexpended. He had ceased work on the project in December and did not expect to resume his research in the coming year because he intends to go to England for a year. He offered to return the unspent balance to the Council for the use of the Committee before the end of March. This offer was gratefully accepted.

(ii) Estimates for 1959-60

During the discussion of the funds which would be required for the year 1959-60, Dr. Macdonald suggested that an increase of \$15,000 be requested. It is anticipated that additional applications for both fellowships and grants will be received as a result of the establishment, this year, of Canada's sixth Dental School at the University of Manitoba.

The number of good Fellowship candidates also appeared to be increasing and in view of the relatively few dental research workers in Canada the Committee considered it imperative to have adequate funds to support highly qualified applicants who show an interest in research. Dr. Marshall pointed out that \$15,000 represented somewhat more than the forecast 20% increase per year which the Council hoped to be able to provide for its Committees and suggested that no specific figure be mentioned in requesting an increase. He assured the Committee that the rate of general increase in funds provided for Council's extramural activities would be reflected in the percentage increase allocated to the Associate Committee on Dental Research.

10. Membership of the Committee

It was noted with regret that the term of Dr. Pagé's appointment would expire on March 31, 1958.

Dr. Aldous also tendered his resignation as a member of the Committee; his term expires in 1959 but he informed the Committee of his intention to spend the coming year at Cambridge and would, therefore, not be in a position to serve the full term of his appointment. His resignation, too, was accepted with regret.

After some discussion as to replacements for Dr. Pagé and Dr. Aldous, and consideration of the fields and geographical areas which such replacements should represent, it was agreed that the Secretary forward to Council the Committee's recommendation that Dr. A. D'Iorio, Department of Physiology, University of Montreal, and Dr. R. L. de C. H. Saunders, Department of Anatomy, Dalhousie University, be appointed to the Committee for a three-year period, ending in 1961.

The Committee also learned that Brig. Wansbrough, who had served on the Committee, ex officio, as the representative of the Royal Canadian Dental Corps, was planning to retire from active duty with the Corps and would thus automatically be replaced on the Committee by his successor. Dr. Macdonald took the opportunity of expressing, on behalf of the Committee, its realization of the great debt owing to Brig. Wansbrough for the long years of service to the committee and assured him that he would be sorely missed.

11. Publications of Grantees

The Secretary reported that during the course of the year, reprints of one paper arising out of work supported by grants from the Committee had been received:

B. E. Walker and F. C. Fraser	The Embryology of Cortisone- induced Cleft Palate	J. Embryol. exp. Morph. 5: 201-209, 1957.
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12. Interdepartmental Consultations

Dr. Barrie raised a question relating to research procedures. He said that investigators sometimes found after analysing teeth that their methods were inadequate. He suggested that there should be a Bureau of Standards which could provide samples and that it be made almost obligatory for anyone to test samples provided by this Bureau before they can obtain support for their work from the Committee. He also suggested that it be made mandatory for those working with histological sections to ask the local professor of pathology for an opinion on his results. He felt that if a pathologist received a small honorarium to cover the expenses involved, he would be glad to undertake to do this. Dr. Paynter suggested that an honorarium in such cases could be regarded as a legitimate charge against a grant.

After brief discussion, the Committee agreed that it could suggest to an investigator that he do this, if it appeared advisable, but it could not make such a consultation mandatory; the Committee is not in a position to tell an investigator how to do research.

13. Special Committee on Government Support of Medical Research

For the information of the Committee, the Chairman mentioned that a Special Committee had recently been set up by the Honourable Gordon Churchill, in his capacity as Chairman of the Privy Council Committee on Scientific and Industrial Research. The purpose of this Committee, of which Dr. Farquharson is Chairman, is to review the procedure by which the Government supports medical research outside its own installations, the adequacy of that support and the ways in which support provided by the various Government agencies should be coordinated.

The Chairman said that the action taken by the new Committee would undoubtedly be of concern to the Associate Committee on Dental Research because there is a great degree of overlap between medical and dental research. He felt that the Associate Committee on Dental Research was in a very good position as far as the new Committee is concerned because Dr. Farquharson is fully acquainted with its activities. The Committee agreed that all possible co-operation should be given the Special Committee.

14. Expression of Appreciation

The Chairman said that he considered the meeting in Montreal to have been a success. All members agreed; it was suggested that while it was necessary to hold most meetings

in Ottawa, the opportunity to hear reports from grantees was most welcome and with this in mind consideration might be given to the possibility of holding a meeting in Toronto at some future date.

The Chairman, on behalf of the Committee, thanked Dr. Lussier most warmly for making such excellent arrangements and for the hospitality extended to the Committee by the University of Montreal.

The Secretary was instructed to write to the University of Montreal, McGill University and Dr. Lussier, to extend the formal thanks of the Committee for their contribution to the success of the meeting.

15. Date of Next Meeting

It was agreed that the next meeting of the Committee be held in Ottawa at approximately the same time in the spring of 1959; the exact date will be set when the date of Council meetings has been decided upon.

The meeting adjourned at 5:30 p.m.

As luck would have it, three members of the Committee were in Ottawa on the date set as the deadline for the receipt of applications (May 10th); Dr. Cope, Dr. Williams and Dr. Wembrough met at the Council on that date, and a preliminary selection of the most suitable applicants was made. In doing so, consideration was given to the letters of the applicants' referees, the geographical location preferred by the applicants in relation to the laboratories in which positions were available, and the desirability of employing students of various years to try to determine what degree of academic training was necessary before a student could derive some benefit from an assistantship. In two cases, where several applicants of more or less equal merit had expressed a preference for the same laboratory, the applicants were subsequently interviewed by the grantees concerned.

Summaries of all applications were then prepared and sent to the members of the Executive. The names of four candidates

APPENDIX "A"

REPORT ON SUMMER UNDERGRADUATE DENTAL RESEARCH ASSISTANTSHIPS

1957 Programme

Following the meeting of the Committee last year, Council approval was obtained, in principle, of the Committee's recommendation that an effort be made to stimulate interest in research among dental undergraduates by offering employment opportunities ("Assistantships") in the laboratories of the Committee's grantees. Six of the grantees who had well-established research programmes were then asked if they would be willing and able to accommodate undergraduates in their laboratories during the summer of 1957. Four of those canvassed were in a position to do so and the funds available to the Committee were just adequate to meet the cost of supplementary grants to permit the employment of this number of undergraduates. Accordingly, on April 18, letters were sent to the Deans of the five Canadian Dental Schools informing them of this new phase of the Committee's programme and inviting them to publicize the plan in their Schools by means of posters which were provided. The Deans of the Dental Schools were unanimous in their approval of the scheme; interest on the part of the students was evident from the fact that a total of 22 applications were received in spite of the late date at which the announcements were distributed. It was interesting to note that student interest was not centred in any one school, nor among students of any one year. Four applicants were students at the University of Montreal, 4 at Toronto, 7 at McGill, 4 at Alberta and 3 at Dalhousie; 10 applicants were completing their first year of Dentistry, 1 his second, and 11 their third.

As luck would have it, three members of the Committee were in Ottawa on the date set as the deadline for the receipt of applications (May 10th); Dr. Copp, Dr. Williams and Brig. Wansbrough met at the Council on that date, and a preliminary selection of the most suitable applicants was made. In doing so, consideration was given to the letters of the applicants' referees, the geographical location preferred by the applicants in relation to the laboratories in which positions were available, and the desirability of employing students of various years to try to determine what degree of academic training was necessary before a student could derive some benefit from an assistantship. In two cases, where several applicants of more or less equal merit had expressed a preference for the same laboratory, the applicants were subsequently interviewed by the grantees concerned.

Summaries of all applications were then prepared and sent to the members of the Executive. The names of four candidates

who appeared to be most suitable were proposed for approval and, on May 31, these four were offered employment. They included one first year student from Alberta to work with Dr. Copp at U.B.C., one first year student from McGill to work with Dr. Leblond at McGill, one second year student from Dalhousie to work with Dr. Aldous at Dalhousie, and one third year student from Toronto to work with Dr. Nikiforuk at Toronto. The salaries paid were \$265. per month for a first year student, \$275. for a second year student, and \$285. for a third year student; these rates were the same as those authorized for science undergraduates employed in the Council's own laboratories during the summer months.

Two letters of varying degrees of protest were received. A professor at one university unfortunately felt that because no assistantship was to be tenable at his institution, this indicated a lack of appreciation for the value of its research programme. Apparently it had not been made clear to the faculty there that assistantships were initially being made available only in the laboratories of grantees of the Dental Committee; a letter of explanation was sent. In addition, an unsuccessful applicant from another university wrote in no uncertain terms to say that in his opinion third year students should be given preference over first year students. He was advised that the Committee was not convinced on this point and had therefore considered it advisable to award the very limited number of assistantships to representatives of all years, on a trial basis.

At the end of the summer, the grantees and students who had participated in this programme were asked for their comments.

Qualifications: To be eligible for appointment as a Medical Research Associate, a candidate must have a doctor's degree in medicine or related science, have shown outstanding competence in his field of study and demonstrated ability to conduct independent research. Under ordinary circumstances, candidates should be under 40 years of age. Applications may be submitted on behalf of Canadians, whether in Canada or abroad, or on behalf of candidates of other nationalities domiciled in Canada with the intention of remaining here.

Salary: The initial salaries of Medical Research Associates will be similar to those paid to National Research Council Research Officers of comparable competence, training and experience, medical training before or after graduation from a Medical School being taken into account as well as research experience. The present starting salary for a new Ph.D. scientist in the National Research Council is \$6,600; allowance may be made for additional experience and proven research ability. Salary payments will be made through the business office of the university

APPENDIX "B"

NATIONAL RESEARCH COUNCIL OF CANADA MEDICAL RESEARCH ASSOCIATES 1958

The National Research Council will continue its contribution to the long term planning and development of medical research in Canadian universities by providing funds for the salaries of a limited number of Medical Research Associates. These may be awarded to individuals of outstanding ability and training who wish to make research a full-time career.

Applications should be made not by the prospective candidate but by the President or Principal of the university (on the recommendation of the Head of the Department concerned and the Dean of the Medical Faculty), who must undertake to provide adequate research facilities and to give the Associate an appropriate honorary or other academic rank on the staff of the Medical School or Faculty, in keeping with the practice of the university. An Associate is not to be regarded as an extramural research worker of the National Research Council.

Tenure: The first appointment of a Medical Research Associate will be for a period of two years. It may be renewed for five-year periods, but not beyond the normal retiring age of the National Research Council or the university concerned. The appointment may be terminated at any time, for good and sufficient reason, by the appointee, the university or the National Research Council.

Qualifications: To be eligible for appointment as a Medical Research Associate, a candidate must have a doctor's degree in medicine or related science, have shown outstanding competence in his field of study and demonstrated ability to conduct independent research. Under ordinary circumstances, candidates should be under 40 years of age. Applications may be submitted on behalf of Canadians, whether in Canada or abroad, or on behalf of candidates of other nationalities domiciled in Canada with the intention of remaining here.

Salary: The initial salaries of Medical Research Associates will be similar to those paid to National Research Council Research Officers of comparable competence, training and experience, medical training before or after graduation from a Medical School being taken into account as well as research experience. The present starting salary for a new Ph.D. scientist in the National Research Council is \$6,600; allowance may be made for additional experience and proven research ability. Salary payments will be made through the business office of the university

and will be subject to income tax. Funds will be provided for the stipend and the contributions made on behalf of the Associate to the university's pension scheme and other benefits in which university staff members normally participate.

Emolument from other sources: A Medical Research Associate may engage in teaching or consulting practice to a limited extent. Routine teaching should be limited, but there need be no restriction in the amount of time spent in the supervision of graduate students, as this activity is considered part of his research programme. Consultations by an Associate in clinical fields must be limited to work related to his research problem. Remuneration may be accepted for these services by an Associate, but the total amount must not exceed \$2,000 per annum.

Research Grant: In the first year of his tenure, the Associate will receive from the National Research Council a grant of \$3,000; in the subsequent years he may apply to the Council or other granting body for funds required to prosecute his research.

Appointment: Recommendations for appointments will be made by a Selection Committee named by the National Research Council. Not more than four appointments will be made in 1958-59. If applications are made by a university on behalf of more than one candidate in a single year, the applicants should be listed in order of preference. The Selection Committee need not be ruled by the university's list, but will be guided by it.

Applications must be made on approved forms, and forwarded by the President or Principal of the university to reach the Awards Office, National Research Council, Ottawa 2, Ontario, not later than 15 February, 1958.

December, 1957.

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| 6 | The therapeutic properties of periodontal cement packs, by W. J. DENTAY and D. C. O'CONNELL. | J. Can. Dent. Assoc. 13: 199-205, 1949. |
| 7 | Phosphatase in saliva, by J. T. DENTAY and J. J. RAE. | J. Dent. Res. 28: 68-71, 1949. |
| 8 | The treatment of acute oral Vincent's infection, by W. J. LINGHORNE and D. F. STEDMAN. | J. Can. Dent. Assoc. July 1949. |
| 9 | A comparison of nine local anaesthetics, by H. S. HAMILTON, B. A. WESTFALL and J. E. W. FERGUSON. | J. Pharmacol. Expt. Ther. 94: 279-307, 1948. |
| 10. | New methods of repairing acrylic dentures without distortion, by D. R. CHRISTIE. | J. Can. Dent. Assoc. 13: 583-590, 1949. |

APPENDIX "C"

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

List of Publications

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
1	New aspects of periodontal research, by H. K. BOX.	Presented at National Convention of Can. Dentists, Toronto May 29 1946 (App. C. 5th Mtg., A.C.D.R., 25 Feb. 1957)
2	Concerning the pathogenesis of periodontal disease, by H. K. BOX.	J. Periodontology 19: 11-20, 1948.
3	The effect of various inorganic salts on the solubility of calcium phosphate, tooth enamel and whole teeth in lactic acid, by J. J. RAE and C. T. CLEGG.	J. Dent. Res. 27: 52-53, 1948.
4	Changes in the calcium and phosphate concentrations of saliva and inorganic salt solutions on shaking with calcium phosphate, by J. J. RAE and C. T. CLEGG.	J. Dent. Res. 27: 54-57, 1948.
5	An improved method for processing acrylic dentures, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 14: 303-317, 1948.
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10.	New methods of repairing acrylic dentures without distortion, by D. R. CHRISTIE.	J. Can. Dent. Assoc. 15: 582-590, 1949.

<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
11	The corrosion of cobalt chromium alloys in commercial cleaning agents, by D. E. R. COGGLES, O. J. WALKER and H. R. MacLEAN.	J. Can. Dent. Assoc. 16: 75-82, 1950.
12	The relation between buffering capacity, viscosity and lactobacillus count of saliva, by J. J. RAE and C. T. CLEGG.	J. Dent. Res. 28: 589-593, 1949.
13	Mineralization of the growing tooth as shown by radiophosphorus autographs (17692), by L. F. BELANGER and C. P. LEBLOND.	Proc. Soc. Expt. Biol. Med. 73: 390-391, 1950.
14	Radio-autographic visualization of bone formation in the rat, by C. P. LEBLOND, C. W. WILKINSON, L. F. BELANGER and J. ROBICHON.	Am. J. Anat. 86: 2, 1950.
15	Fluoride studies related to the human diet, by MARY P. HAM and M. DOREEN SMITH.	Can. J. Research, F, 28: 227-233, 1950.
16	Studies in the regeneration and re-attachment of supporting structures of the teeth. I. Soft tissue re-attachment, by W. J. LINGHORNE and D. C. O'CONNELL.	J. Dent. Res. 29: 419-428, 1950.
17	An examination of the fluoride content of teeth from some Ontario districts, by SHIRLEY R. HARNESS and M. DOREEN SMITH.	J. Can. Dent. Assoc. Jan. 1951.
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<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
22	Studies in the regeneration and re-attachment of supporting structures of the teeth. II. Regeneration of alveolar process, by W. J. LINGHORNE and D. C. O'CONNELL.	J. Dent. Res. 30: 604-614, 1951.
23	Radioactive isotopes in dental research, by J. P. LUSSIER and A. D'IORIO.	The Brit. Dent. Annual, 1952.
24	Determination of fluoride in water by the aluminum-hematoxylin method, by MARGARET J. PRICE and O. J. WALKER.	Anal. Chem. 24: 1953, 1952.
25	Autoradiographic detection of S^{35} in the membranes of the inner ear of the rat, by L. F. BELANGER.	Science 118: 520-521, 1954.
26	The motile non-sporulating anaerobic rods of the oral cavity, by J. B. MACDONALD.	University of Toronto Press, Toronto, 1953.
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<u>Paper No.</u>	<u>Title and Author</u>	<u>Reference</u>
31	Autoradiographic visualization with Ca^{45} of normal growth of the incisor of pigs and the effect of fluorine feeding, by L. F. BELANGER, W. E. LOTZ, W. J. VISEK and C. L. COMAR.	Anat. Rec. 119: 53-70, 1954.
32	Fluorine balance studies of four infants, by MARY P. HAM and M. DOREEN SMITH.	J. Nutrition 53: 215-223, 1954.
33	Fluorine balance studies of three women, by MARY P. HAM and M. DOREEN SMITH.	J. Nutrition 53: 225-232, 1954.
34	The effect of propylthiouracil on the development of molar teeth, by K. J. PAYNTER.	J. Dent. Res. 33: 364-376, 1954.
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36	Autoradiographic visualization of Ca^{45} intake by normal and pathological cartilage <u>in vitro</u> , by L. F. BELANGER.	Proc. Soc. Expt. Biol. & Med. 88: 150-152, 1955.
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38	Ammonia production in saliva. I., by J. J. RAE, H. M. SHELILT and C. T. CLEGG.	J. Dent. Res. 34: 886-888, 1955.
39	Growth in width of the head of the Macaca rhesus monkey as revealed by vital staining, by A. H. CRAVEN.	Am. J. Orthodontics 42: 341-362, 1956.
40	Preparing single or serial section of calcified bones and teeth, by B. N. KROPP.	Stain Tech. 31: 253-261, 1956.

Paper No.	Title and Author	Reference
41	The effects of single injections of soluble components of "fusospirochetal" materials in experimental animals, by H. A. HUNTER and J. B. MACDONALD.	J. Dent. Res. 35: 4-8, 1956.
42	Mixed infections of the oral cavity, by J. B. MACDONALD.	J. Norwegian Dent. Assoc. April 1956.
43	The pathogenic components of an experimental fusospirochetal infection, by J. B. MACDONALD, R. M. SUTTON, M. L. KNOLL, E. M. MADLENER and R. M. GRAINGER.	J. Infectious Dis. 98: 15-20, 1956.
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45	The embryology of cortisone-induced cleft palate, by B. E. WALKER and F. C. FRASER.	J. Embryol. exp. Morph. 5: 201-209, 1957.

Representing the Dental Profession

5. Dr. G. Castaldi,
Faculty of Dentistry,
University of Alberta,
Edmonton, Alberta
6. Dr. C. D. Husband,
Suite 110, Metro Block,
Red Deer, Alberta
7. Dr. Jean-Paul Lussier,
Faculty of Dentistry,
University of Montreal,
Montreal, P. Q.
8. Dr. R. J. O'Neill,
2525 Pine Street,
Vancouver, B. C.

* Member of Executive.

APPENDIX "D"

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment

	<u>Term to expire</u>
★ 1. Dr. D. H. Copp, <u>Chairman</u> , Department of Physiology, University of British Columbia, Vancouver, B. C.	31 March 1959
2. Dr. E. W. R. Steacie, <u>ex officio</u> President, National Research Council, Ottawa 2, Ontario	- - -
3. Dr. R. F. Farquharson, <u>ex officio</u> Vice-President (Medical), National Research Council, Ottawa 2, Ontario	- - -
4. Dr. D. L. Thomson, <u>ex officio</u> Vice-Principal, McGill University, Montreal, P. Q.	- - -
<u>Representing the Dental Profession</u>	
5. Dr. C. Castaldi, Faculty of Dentistry, University of Alberta, Edmonton, Alberta	31 March 1960
6. Dr. C. D. Husband, <u>ex officio</u> Suite 110, Metro Block, Red Deer, Alberta	31 March 1959
★ 7. Dr. Jean-Paul Lussier, Faculty of Dentistry, University of Montreal, Montreal, P. Q.	31 March 1960
8. Dr. R. J. O'Neil, 2525 Pine Street, Vancouver, B. C.	31 March 1959

★ Member of Executive.

Term to expire

Representing the Dental Profession (cont'd)

- | | | |
|------|---|---------------|
| ★ 9. | Dr. A. G. Racey,
Faculty of Dentistry,
McGill University
Montreal, P. Q. | 31 March 1959 |
| 10. | Dr. C. H. M. Williams,
Associate Professor of Periodontology,
Faculty of Dentistry,
University of Toronto,
Toronto 5, Ontario | 31 March 1960 |

Representing the Royal Canadian Dental Corps,
Department of National Defence

- | | | |
|-------|--|-------|
| ★ 11. | Brig. E. M. Wansbrough, <u>ex officio</u>
Director General of Dental Service,
Royal Canadian Dental Corps,
Department of National Defence,
Ottawa, Ontario | - - - |
|-------|--|-------|

Representing the Division of Dental Health,
Department of National Health and Welfare

- | | | |
|-----|---|-------|
| 12. | Dr. H. K. Brown, <u>ex officio</u>
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ontario | - - - |
|-----|---|-------|

Representing Dental Research,
Department of Veterans Affairs

- | | | |
|-----|--|-------|
| 13. | Dr. L. A. Kilburn, <u>ex officio</u>
Director of Dental Research,
Department of Veterans Affairs,
Ottawa, Ontario | - - - |
|-----|--|-------|

Representing the Basic and Medical Sciences

- | | | |
|-----|---|---------------|
| 14. | Dr. J. G. Aldous,
Department of Pharmacology,
Dalhousie University,
Halifax, N. S. | 31 March 1959 |
|-----|---|---------------|

Term to expireRepresenting the Basic and Medical Sciences (cont'd)

- | | | |
|-------|--|---------------|
| 15. | Dr. H. J. Barrie,
Department of Pathology,
University of Toronto,
Toronto 5, Ontario | 31 March 1960 |
| * 16. | Dr. J. M. R. Beveridge,
Department of Biochemistry,
Queen's University,
Kingston, Ontario | 31 March 1959 |
| 17. | Dr. J. B. Macdonald,
Director,
Forsyth Dental Infirmary for Children,
140 The Fenway,
Boston 15, Mass. | 31 March 1960 |
| 18. | Dr. K. J. Paynter,
Division of Dental Research,
University of Toronto,
Toronto, Ontario | 31 March 1960 |
| 19. | Dr. Edouard Pagé,
Department of Biology,
University of Montreal,
Montreal, P. Q. | 31 March 1958 |
| 20. | Dr. G. D. Scott,
Department of Physiology,
University of Toronto,
Toronto, Ontario | 31 March 1960 |
| 21. | Miss D. J. Wright, <u>Secretary</u> ,
National Research Council,
Ottawa 2, Ontario | - - - |

* Member of Executive.

INITIAL DISTRIBUTION

(i) To members of the
Associate Committee on Dental Research

- | | |
|------------------------------------|---|
| 1. Dr. D. H. Copp, <u>Chairman</u> | 12. Dr. R. J. O'Neil |
| 2. Dr. J. G. Aldous | 13. Dr. E. Page |
| 3. Dr. H. J. Barrie | 14. Dr. K. J. Paynter |
| 4. Dr. J. M. R. Beveridge | 15. Dr. A. G. Racey |
| 5. Dr. H. K. Brown | 16. Dr. G. D. Scott |
| 6. Dr. C. Castaldi | 17. Dr. E. W. R. Steacie |
| 7. Dr. R. F. Farquharson | 18. Dr. D. L. Thomson |
| 8. Dr. C. D. Husband | 19. Brig. E. M. Wansbrough |
| 9. Dr. L. A. Kilburn | 20. Dr. C. H. M. Williams |
| 10. Dr. J.-P. Lussier | 21. Miss D. J. Wright, <u>Secretary</u> |
| 11. Dr. J. B. Macdonald | |

(ii) To other persons:-

22-27 Deans of Dental Schools (who are not members of the Committee)

- | | | |
|---------------|---|--------------------|
| 22. Alberta | - | Dr. W. S. Hamilton |
| 23. Dalhousie | - | Dr. J. D. McLean |
| 24. Manitoba | - | Dr. J. W. Neilson |
| 25. McGill | - | Dr. J. McCutcheon |
| 26. Montreal | - | Dr. P. Geoffrion |
| 27. Toronto | - | Dr. R. G. Ellis |

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-FIFTH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

FEBRUARY 26 - 27, 1959

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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NATIONAL RESEARCH COUNCIL

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February 26 and 27, 1958

Initial Distribution

The first session convened at 9:30 a.m. on February 26th in the Council Chamber of the National Research Council, Ottawa

1. Attendance

Dr. D.B. Copp, Chairman
Dr. H.J. Barrie
Dr. J.M.R. Beveridge
Dr. H. Brown
Dr. C. Castaldi
Dr. A. D'Iorio
Dr. L.A. Kilburn
Dr. J.-P. Lussier
Dr. J.B. MacDonald
Dr. K.J. Paynter
Dr. A.G. Racey
Dr. S.L. de C.E. Saunders
Dr. G.D. Scott
Col. G.B. Shillington (for Brig. K.W. Baird)
Miss D.J. Wright, Secretary

By Invitation

Dr. L.F. Belanger
Dr. L. Francis
Dr. H.M. Madlener
Dr. J.W. Neilson
Dr. G. Nikiforuk

Secrets were received from:

Dr. G.D. Husband
Dr. H.J. G'Neil
Dr. D.L. Thomson
Dr. C.R.M. Williams

NATIONAL RESEARCH COUNCIL

Proceedings

of the Twenty-fifth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

February 26 and 27, 1958

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Dr. L.F. Belanger
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Dr. G. Nikiforuk

Regrets were received from:

Dr. C.D. Husband
Dr. R.J. O'Neil
Dr. D.L. Thomson
Dr. C.H.M. Williams

2. Chairman's Remarks

The Chairman welcomed the new members of the Committee, Dr. A. D'Iorio of the Department of Physiology, University of Montreal, and Dr. R.L. de C.H. Saunders, Department of Anatomy, Dalhousie University. He also introduced the guests of the Committee, Dean J.W. Neilson of the Faculty of Dentistry at the University of Manitoba, and Dr. Belanger, Dr. Francis, Dr. Madlener and Dr. Nikiforuk who had been invited to report on their work.

The Chairman commented on the success of the twenty-fourth meeting of the Committee in Montreal and hoped that it would, on future occasions, be able to hold other meetings in association with one or other of the dental schools.

The Chairman informed the Committee that the budget had been increased to \$80,000 for the coming year 1959-60; the Committee should therefore be in a position to provide fairly adequate support for the worthwhile projects for which funds had been sought.

3. Minutes of the Twenty-fourth Meeting

It was MOVED by Dr. Paynter and SECONDED by Dr. Kilburn THAT the Minutes of the Twenty-fourth Meeting be taken as read, and approved.

CARRIED

4. Business Arising from the Minutes

The Chairman reported that the funds encumbered at the Twenty-fourth Meeting to permit the continued employment of Dr. Weiss under grant D.A. 61 for a three-month period, and to provide for Summer Dental Research Assistantships under grants D.A. 23, D.A. 69, D.A. 70, D.A. 71 and D.A. 72 had been released by the Executive.

5. Presentation of Progress Reports

(a) Dr. G. Nikiforuk, University of Toronto

Dr. Nikiforuk reviewed his recent work on the organic constituents of dental tissues. He pointed out that dental tissue offers interesting opportunities for the study of the mechanisms of calcification because of its peculiar properties: it is the only composite tissue that becomes mineralized and is the only tissue that has constancy in its organic elements.

Following an outline of the methods used in his study which, while supported initially by the Associate Committee, now receives its major support from the U.S. Public Health Service, Dr. Nikiforuk described the results he has obtained to date with the aid of paper chromatography. He reported that in view of his findings he now doubts that the major component of the organic enamel fraction is actually keratin, as was previously supposed, because its amino acid character is not that of keratin. Following further elaboration of his results, Dr. Nikiforuk said that he hopes soon to be able to apply radioisotope techniques in his work, for only in this way will he be able to overcome the difficulties inherent in the study of such small amounts of experimental material as are now available to him.

To Dr. Beveridge's question regarding the total amino acid sulphur in the protein present in enamel, Dr. Nikiforuk replied that he had not done any quantitative work on this. He referred to recent work by Belanger using radioactive cystine which indicated that there was very little; he expressed some doubt as to the value of a sulphur analysis, however, since one could not tell where it comes from.

Dr. Beveridge inquired whether an attempt had been made to identify cystine or cysteine on the chromatograms. Dr. Nikiforuk said that he had tried to do this but had not got a good enough spot to say whether or not cystine could be identified.

In thanking Dr. Nikiforuk for his presentation, the Chairman pointed out that little is yet known about the nature of the matrix and basic work of this kind is very important to the eventual understanding of the mineralization of organic matrix.

(b) Dr. A.S.V. Burgen. D.A. 61 (Appendix A)

Following presentation of Dr. Burgen's report on the secretion of protein and of bicarbonate ions in saliva, Dr. Beveridge expressed the opinion that further knowledge of the conditions affecting salivary secretion will contribute greatly to the solution of problems in the field of dentistry, and said that Dr. Burgen is to be congratulated on the calibre of his work.

(c) Dr. K.I. Melville. D.A. 66 (Appendix F)

Dr. Francis reported to the Committee on studies on the mechanism of gingival changes associated with drug administration. This work had been done under Dr. Melville's direction.

With the aid of slides, he illustrated the effects on the gingiva of injecting varying amounts of dilantin into different animals, alone and in combination with antihistaminic agents.

The studies have indicated that pyridoxine in combination with dilantin may have some effect in preventing hyperplasia, and further experiments are planned to elucidate this. In addition to the animal investigations, Dr. Francis reported that he had treated a patient with hyperplasia with amounts of 50 mg. of pyridoxine per day and his condition seems to have improved.

Dr. Barrie congratulated Dr. Francis on a very interesting piece of work. He offered to send Dr. Francis some gingiva obtained from autopsies if it would assist him in the histological aspects of his study, and suggested that it might be worthwhile to do stains to determine the presence of mast cells.

(d) Dr. L.F. Belanger. D.T. 52 (Appendix L)

Dr. Belanger described in some detail his recent studies on lathyrism in animals and illustrated his presentation with autoradiograms. He reported that he had been able to reproduce a condition in which there is precipitation of round crystalline masses containing Ca^{45} in the vicinity of normal mineralized tissue.

Asked about the part played by some local factors in mineralization, Dr. Belanger said that mineralization depended on the organic constituents which form bone and cartilage and also on local cytology; as osteoblasts, amonoblasts, etc. are damaged, demineralization may be the result of something going wrong in the synthesis of collagen so that not enough is formed, or possibly some factor modifies the adjacent environment. He pointed out that mineralization takes place in areas rich in acid polysaccharides: demineralization occurs in areas which are poor in neutral polysaccharides.

Dr. Copp said that a relationship between collagen and the properly ordered calcification of tissues would have very interesting theoretical implications.

Dr. Paynter expressed surprise that Dr. Belanger's pictures indicated that there is demineralization of material that has already been demineralized. Dr. Barrie asked if this decrease in substance is due to a lack of formation or to a greater loss of material than is normal, occurring in those tissues which are continually replacing themselves. Dr. Belanger said that it was hard to tell.

After complimenting Dr. Belanger on his work, Dr. Nikiforuk added that the advantage of autoradiographic methods is that one can study young, growing tissue whereas chromatographic methods necessitate working with old tissues that may not be good. Dr. Nikiforuk suggested that the fact that neutral polysaccharides are PAS positive and acid mucopolysaccharides are not, might be due to the masking effects of acid mucopolysaccharides rather than solvation.

Dr. Copp said that since the main defects in animals with lathyrism appear to be in the matrix, it might be interesting to study the changes in the matrix with respect to the various components.

(e) Dr. C.P. Leblond. D.A. 23 (Appendix B)

Following Dr. Saunders' presentation of Dr. Leblond's report on the uptake of labelled methionine by dentin and enamel matrix, there was some discussion of the compounds used.

Dr. Nikiforuk said that if radioactive amino acid is injected into animals and undergoes metabolic changes, or a turn-over takes place, the pattern should be the same regardless of the label. He added that radioautography is an excellent tool in work of this type since it makes possible the study of dynamic tissue which is actually in the process of being formed.

Dr. Belanger said that Dr. Leblond was to be congratulated on his work, of which the current project is only one facet; he felt that Dr. Leblond's conclusions must be accepted, since he had obtained the same results with so many different labelled substances.

Dr. Beveridge suggested that the whole process might be based on the deposition of cystine, to which Dr. Belanger replied that even if this were true it would still be a process of protein synthesis.

Dr. Nikiforuk said that if it were a matter of protein synthesis and nothing else, it is difficult to understand why one would have diffusion.

(f) Dr. E.M. Madlener. D.A. 71 (Appendix E)

Dr. Madlener described in some detail his studies of the past year on the four pathogenic components of exudate H.G. and spirochetes.

Dr. Macdonald pointed out that unlike working with Escherichia coli, work with these organisms presented great technical difficulties because they grow slowly and their anaerobic and nutritional requirements are not yet fully understood. He reported that he had recently been cultivating spirochetes by excluding oxygen and growing them in a nitrogen atmosphere. Since this approach might solve some of Dr. Madlener's difficulties, he offered to cultivate any spirochetes which Dr. Madlener might send him.

Dr. Macdonald also referred to biochemical studies by Gibbons; the organism requires a growth factor and Gibbons had found that by using an unsaturated fatty acid with a fairly high molecular weight (1 g/ml.) the organism can be grown in the absence of agar.

Dr. Barrie said that he was impressed by the care taken by Dr. Madlener in his bacteriological work. He suggested that Dr. Madlener conclude his present investigation during the coming year and apply the tagged antibiotic technique to studies of bacteriological problems in the mouth.

(g) Dr. D.H. Copp. D.T. 59

Dr. Copp reviewed the work carried out during the year on the factors affecting calcium deposition in and removal from bones and teeth. The studies were carried out with radioactive calcium on adult animals where there was no stress of growth, and consequently no drop in the blood phosphate and no change in the bone kinetics. In parathyroidectomized animals, there was a sharp drop in new bone formation and a slight drop in resorption; the growth of bone was slowed down although the bone remained well mineralized. There was, however, a significant decrease in absorption of calcium from the intestinal tract. Dr. Copp referred to a report by Talmadge which indicated that the parathyroid hormone may effect the absorption of minerals. In general, one can conclude that bone is very much involved in the homeostatic control of phosphate in the body whereas teeth, because of the lack of vessels or because of the nature of the odontoblasts, do not participate in normal mineralization.

To Dr. Lussier's question regarding the state of his experimental animals with respect to vitamin D, Dr. Copp said that they had adequate vitamin D in their diets (400 units /kg). He added that even without vitamin D, however, they had appeared to be no more rachitic.

Dr. Copp referred to discussions he had had with Dr. Bauer regarding the accretion rate, after which it had been

concluded that the figure given by Bauer for accretion represents not only new bone formation but also the slow diffusion of calcium into existing crystals. Talmadge and Roland at the Argonne Laboratory had agreed that about 50% of the accretion is merely slow diffusion.

Dr. Nikiforuk noted that Leblond had reported very good evidence of the stability of the organic fraction of dentin, and the same thing appears to be true of the inorganic fraction.

In adult animals injected with radioactive phosphate radioactivity is detected in large quantities on the pulpal ending of enamel. Dr. Nikiforuk felt that the suggestion that the inorganic fraction of this tissue is constant, was not logical.

Dr. Copp said that work with radioactive calcium in this area was difficult. He pointed out that the inorganic material may be relatively stable. He felt that the essential difference between dentin and bone is the constant digging out of bone, with change in calcium content. In the case of teeth, this does not occur except in special conditions such as resorption of deciduous teeth.

(h) Dr. J.-P. Lussier. D.A. 70 (Appendix D)

Dr. Lussier reported on the work done on hormonal factors and their relationship to citrate metabolism in bones and teeth.

During the discussion which followed, Dr. Copp noted that there is not universal agreement as to the action of vitamin D and only further experimentation will produce the answer to this question.

Dr. Belanger inquired about the cellular source of citric acid. Dr. Copp said that citric acid, being part of the carbohydrate cycle, is produced in large amounts in all cells of the body. However, very little of this citric acid escapes into the blood. He pointed out that bone is in a unique position; it contains about 90% of the citric acid in the body but most of it is biologically inert. Neuman has shown that if you analyse marrow blood for citrate, the citrate concentration is higher than that in arterial blood. The problem of bone citrate is something quite apart from the general use of citrate as part of the carbohydrate cycle and is probably related to the specific action of bone cells.

With respect to the study of tooth movement, Dr. Castaldi pointed out that the rotation of a tooth in orthodontic procedures is very difficult because it tends to return

(i) Dr. K.J. Paynter. D.A. 64 (Appendix G)

Dr. Paynter outlined the studies on the nature of cementum during the past year. He described the difficulty encountered in preparing sections of the jaws of 15 day-old rats but indicated that these had already been overcome.

In connection with the autoradiographic techniques used, Dr. Copp pointed out that thymidine labelled with tritium is a thousand times more toxic than tritiated water and toxic effects are obtained at what appear to be experimentally low levels.

Dr. Paynter said that he hoped to conclude this investigation during the coming year.

(j) Dr. K.J. Paynter and Dr. A.M. Hunt. D.A. 72
(Appendix H)

Dr. Paynter reported progress made by himself and Dr. Hunt on the relation between nutrition to morphology and caries-susceptibility in rats.

Dr. Castaldi asked if there were more or less malocclusion when fluoride was used in the rat diets. Dr. Paynter replied that there was less evidence of malocclusion but was unable to explain the reason for this. Dr. Macdonald noted that the teeth of rats with high phosphate diet had apparently been smallest and most susceptible to caries; this finding was contrary to that of Sobel who had reported that high phosphate diet led to teeth less susceptible to caries. Dr. Paynter suggested that this may have been due to the fact that Sobel did not reduce the calcium content of the diet, as he had done.

(k) Dr. R.L. Tegart. D.A. 73 (Appendix I)

Dr. Castaldi presented the report submitted by Dr. Tegart on his study of inflammatory response to sterile glass cannulae in connective tissue. It was noted that the project was progressing satisfactorily and Dr. Tegart had sufficient funds left from his original grant to conclude the investigation.

(l) Dr. R.D. Haryett. D.A. 69 (Appendix J)

Dr. Paynter presented the report of Dr. Haryett on his work on traumatic tooth movement and the subsidiary investigation of basic oral reflexes using decerebration techniques.

With respect to the study of tooth movement, Dr. Castaldi pointed out that the rotation of a tooth in orthodontic procedures is very difficult because it tends to return

to its original position. If it could be shown that a tooth can be rotated with forceps, the time of treatment would generally be reduced substantially.

(m) Dr. K.A. McMurchy. D.A. 67 (Appendix K)

Dr. Paynter reported that he had reviewed Dr. McMurchy's report and found it entirely satisfactory. It was noted that no further funds were being requested in support of this investigation.

(n) Dr. W.J. Linghorne. D.A. 22 (Appendix C)

Dr. Barrie presented a concise report on the work of Dr. Linghorne to date.

6. Meeting of the Executive

The Chairman reported that a meeting of the Committee's Executive had been held on the evening of February 26th. All applications for Fellowships and grants had been reviewed and the Executive's recommendation was put before the Committee as each application was considered.

7. Applications for Fellowships, 1959-60

Prior to considering individual applications, the question of stipends was reviewed. The Chairman said that Fellowship stipends, generally speaking, are low in relation to the higher costs of living and tuition, and competitive opportunities. The current stipend for Graduate Dental Research Fellowships offered by the National Research Council ranges from \$2,300 to \$4,500 per annum. Senior Dental Research Fellowships may have a stipend of up to \$5,000 per annum.

It was MOVED by Dr. Beveridge and SECONDED by Dr. Macdonald THAT the upper limit of the Graduate Dental Research Fellowships be increased to \$5,000 per annum and that the classification of Senior Dental Research Fellowship be dropped.

CARRIED

Five applications for Fellowships were carefully considered and the following recommendations made:

Blackmore, R.V.	To work in the Dept. of Bacteriology, Rochester School of Medicine and Dentistry, under Dr. H.R. Morgan. (Renewal)
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APPROVED \$4,500

Dale, J.G.

To work in the Harvard School of Dental Medicine and the Forsyth Dental Infirmary, under Dr. J.T. Irving. (Renewal)

APPROVED \$4,500

Johnston, M.C.

To work in the Dept. of Dentistry and Dental Research, University of Rochester, under Dr. E. Johansen, beginning January, 1960.

APPROVED \$2,500 for six-months period

Slapcoff, E.

To work in the Dept. of Oral Surgery, Northwestern University, under Dr. O. Stuteville.

NOT APPROVED

Socransky, S.S.

To work in the Harvard School of Dental Medicine and the Forsyth Dental Infirmary, under Dr. J.B. Macdonald. (Renewal)

APPROVED \$4,500

8. Applications for Grants, 1959-60

Consideration was given to 18 applications for grants totalling \$54,685. In addition, it was noted that the second annual instalments, totalling \$9,800, of term grants awarded last year in support of the work of Dr. Copp and Dr. Belanger would be paid in 1959-60.

The following recommendations were made with respect to applications:

<u>Applicant</u>	<u>Title of Project and Amount Requested</u>	<u>Recommendation</u>
Burgen, A.S.V., Physiology, McGill	Studies on protein secretion and other processes in the salivary glands. D.A. 61 \$ 6,610.00	Three-year term grant of \$6,000 per annum.
Castaldi, C.R., Pedodontics, Alberta	A study of enamel faults and its relationship to the time of cerebral damage. \$ 2,625.00	Equipment grant of \$885 and annual block grant of \$1,500 for projects of Dr. Castaldi and Dr. McMurchy.

<u>Applicant</u>	<u>Title of Project and Amount Requested</u>	<u>Recommendation</u>
Francis, L.E., Clinical Dent- istry, McGill	Studies on gingival changes due to drug administration. \$ 5,100.00	\$ 5,100.00
Hart, H.W. and Hildes, J.A., Dentistry, Manitoba	Dental Health aspects of Arctic natives. \$ 1,650.00	\$1,650.00
Hoffman, A.E., Oral Surgery, Dalhousie	The use of an anorganic bone graft following partial mand- ibular resection in the rat. \$ 2,350.00	\$2,350.00
Kleinberg, I., Biochemistry, Manitoba	1. Further studies on the role of the dental plaque in the caries process. 2. Further studies on the effect of substances re- moved during the refining of carbohydrates on calcium phosphate solubility. \$ 4,685.00	Equipment grant of \$545 and annual grant of \$3,800. Amount requested for travel not approved.
Leblond, C.P., Anatomy, McGill	Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements. D.A. 23 \$ 3,850.00	\$3,850.00
Linghorne, W.J. Medical Res., Toronto	An investigation of the mechanisms in the mainten- ance and in the repair of the supporting structures of the teeth. D.A. 22 \$ 2,500.00	Not approved
Lussier, J.-P., Physiology, Montreal	Studies on citrate metab- olism in bone and teeth as affected by hormonal and nutritional factors. D.A. 70 \$ 4,850.00	Three-year term grant of \$4,800 per annum.

<u>Applicant</u>	<u>Title of Project and Amount Requested</u>	<u>Recommendation</u>
Madlener, E.M., Dental Research, Toronto	The characterization of an experimental "Fusospiro- chetal" lesion. D.A. 71 \$ 4,700.00	\$4,700.00
	It was agreed that Dr. Macdonald write to Dr. Madlener suggesting a programme for the termin- ation of this project during the coming year and encouraging him to apply next year for support for a different project.	
McMurchy, K.A., Dentistry, Alberta	A study of anoxic damage on the teeth of rats. \$ 700.00	See Castaldi
McMurchy, K.A., Dentistry, Alberta	A study of the effect of Vitamin E deficiency on the teeth of rats. \$ 700.00	See Castaldi
Mezl, Z., Oral Pathology, Montreal	Penetration of minerals from saliva and shifting of minerals in the carious lesion. \$ 2,000.00	Equipment grant of \$1,500 and annual grant of \$500
Nikiforuk, G., Dental Research, Toronto	"Profexray" (Grentz ray) model TX-10 with range of 3 to 17 KVP \$ 1,500.00	Equipment grant of \$1,500.00
Paynter, K.J., Oral Anatomy, Toronto	Investigations into the nature of cementum. D.A. 64 \$ 750.00	\$750.00
Paynter, K.J. & Hunt, A.M., Oral Anatomy, Toronto	The relation of nutrition to morphology and caries susceptibility in the teeth of rats. D.A. 72 \$ 5,000.00	Three-year term grant of \$5,000 per annum

<u>Applicant</u>	<u>Title of Project and Amount Requested</u>	<u>Recommendation</u>
Perreault, J.G. & Donohue, W.B., Dentistry, Montreal	The effects of a heavy dose of roentgen rays on the developing structures of the skull in young cats. \$ 4,050.00	\$4,000.00
Smith, M.D., Food Chemistry, Toronto	Absorption and retention of fluoride in human teeth (dentin and enamel) from long term exposure to high and low dietary fluoride levels. \$ 2,670.00	Not approved

9. Summer Dental Research Assistantships, 1959

Summer employment opportunities in the research laboratories of the Committee's grantees were again advertised by means of posters distributed to the six Canadian dental schools. Twenty-three applications were received: 10 from third-year students, 9 from second-year students and 4 from first-year students. All dental schools were represented among the applicants: 4 from Toronto, 4 from Montreal, 5 from McGill, 7 from Alberta, 2 from Dalhousie and 1 from Manitoba. Summaries of all applications (See Appendix M) were circulated to the Committee.

For the benefit of those who were unfamiliar with this training scheme, the Chairman said that these Assistantships had been inaugurated three years ago to get good dental students interested in research in the hope that some at least would eventually become career investigators. Applications are submitted directly to the Secretary of the Committee, although it is necessary for the applicant to have the approval of the research investigator with whom he hopes to work.

It was agreed that this procedure should be continued for a few years because of its publicity value; when the scheme has become firmly established it may be simpler for the grantees of the Committee to include, in their grant applications, provision for the employment of dental undergraduates in their laboratories.

In view of the funds available to the Committee, it was agreed that, in general, Assistantships be offered for a period of three months, rather than for four months as heretofore,

unless specifically requested for longer by the grantee in question. Dr. Paynter suggested that, if some successful applicants were to be available for a longer period, the Canadian Dental Association might consider providing the necessary funds to pay them for a fourth month.

Following assessment of the qualifications of the applicants, the following recommendations were made:

Andreas, F.P., Univ. of Toronto	To work with Dr. Paynter for three months @ \$305 (Renewal)	\$ 915
Kirby, P.J., Univ. of Alberta	To work with Dr. Haryett for 3½ months @ \$305 (Renewal)	\$1,065
Levine, M., Dalhousie Univ.	To work with Dr. Hoffman for four months @ \$305	\$1,220
Marcil, A., Univ. of Montreal	To work with Dr. Lussier for three months @ \$285	\$ 855
Paszti, M., Univ. of Toronto	To work with Dr. Madlener for three months @ \$305	\$ 915
Trester, P.H., Univ. of Manitoba	To work with Dr. Kleinberg for 3½ months @ \$265	\$ 930
Weinlander, G.H.G., McGill Univ.	To work for Dr. Leblond for three months @ \$285	\$ 855
		\$6,755

Supplementary grants in the amounts indicated will be provided to the grantees who will act as supervisors of the above students to cover the cost of their salaries, if the successful applicants accept the Assistantships.

It was further agreed that if funds can be found, an Assistantship be offered to Mr. M. Wasylchuk, third year student at the University of Alberta, to work in the laboratory of Dr. Copp at the University of British Columbia for a period of three months.

Dr. Neilson inquired about the feasibility of encouraging several dental students from any one School to apply for Assistantships when funds are so limited. The Chairman said that the number of Assistantships awarded each year depends

both on the funds available and on the availability of work in the grantees' laboratories. He agreed with Dr. Macdonald that while individual students should perhaps not be encouraged to apply, it is desirable to bring the Assistantships to the attention of the classes as a whole. The competitive quality of the Assistantships was held to be desirable.

Dr. Paynter said that at the University of Toronto, applications are screened internally by the Faculty's research committee and only the best applicants who can be assured of accommodation in the Faculty's laboratories are encouraged to apply to the Committee.

10. Financial Status of the Committee

The Chairman reported that after paying the cost of the meeting, slightly over \$4,000 would remain at the disposal of the Committee for the year 1958-59.

He also informed the Committee that its allocation for the year 1959-60 had been increased to \$80,000.

After reviewing the grants, fellowships and assistantships recommended for approval, it was agreed that the four equipment grants to Dr. Castaldi, Dr. Kleinberg, Dr. Mezl and Dr. Nikiforuk, totalling \$4,430, be paid from the unexpended portion of the Committee's 1958-59 allotment.

The following commitments were therefore made against the 1959-60 allotment:

4 term grants	\$ 20,600
11 annual grants	33,200
4 fellowships	16,000
7 assistantships	6,755
Cost of 26th meeting	3,000
Contingencies	445

\$ 80,000

11. Dental Research Programme at McGill University

The Chairman invited Dr. Francis, who had recently been appointed to the full-time staff of the Faculty of Dentistry at McGill, to outline the Faculty's plans for dental research.

12. Dental Dr. Francis said that in the past very little dental research had been done by dental men at McGill although some work in this field had been undertaken by medical investigators in the basic science departments of physiology, pharmacology and histology. These investigators had often shown a certain reluctance to accept dental men in their departments for research training and as a result there had been little opportunity for dental graduates to work toward further degrees or to obtain guidance in research.

This, however, is now changing. In the new medical centre, for instance, the Department of Pharmacology has set aside three rooms for dental research, which will be equipped by the University for this purpose. Dr. Francis felt that as good men are found, other departments too will open their doors to dental graduates, and it was his hope that they would be accepted not as "lame ducks", but as useful participants in the research and teaching programmes.

A nucleus of dental men who are interested in research is being built up in the Faculty of Dentistry. Space is being provided, and equipment and a certain amount of money is available for their research projects. Problems relating to oral flora, for instance, have been discussed with members of the Department of Bacteriology at the Montreal General Hospital; the virologists are interested in co-operating in work of this type, and many patients with viral infections will be available for study.

Dr. Francis said that the new group engaged in dental research at McGill does not intend to make application for grants in aid for the first year or two. When good men have proved themselves, and the programme has been established, that will be the time to apply to the granting bodies for support.

The Faculty of Dentistry at McGill has now set up a committee on graduate studies and research. It is hoped that as the dental research programme develops, dental students can be exposed early to the stimulation provided by research and will be encouraged to take further training in the basic sciences after receiving their degrees in Dentistry.

Dr. Francis felt that in view of the new enthusiasm now evident among members of the Faculty, and the interest being shown by students of the senior years, the future for dental research at McGill seems bright. He looked forward to worthwhile contributions being made in this field by the Faculty of Dentistry within the next five years.

12. Dental Research Programme at University of Manitoba

The Chairman called upon Dr. Neilson, Dean of the new Faculty of Dentistry at the University of Manitoba, to report on the programme and facilities which are being developed there.

Dr. Neilson told the Committee that, as a clinician, he was very interested in fundamental research and hoped that a suitable programme could be developed in his Faculty as time goes on. At the present time, staff is being recruited and it is expected that eventually there will be 12 or 13 full-time members in the clinical field and 7 full-time in the fundamental aspects of dentistry. An attempt will be made to limit the teaching load of full-time staff to twelve hours per week, so that there will be an opportunity for them to undertake research.

Dr. Neilson paid tribute to the valuable contribution made to his planning by Dr. Paynter's survey of dental teaching and research in Manitoba; Dr. Macdonald's report on British Columbia had also been of considerable interest to him.

One of Dr. Paynter's recommendations had been the establishment of bursaries. At the present time, four bursaries are being held - in Alabama, Pittsburgh, Illinois and Indiana - and while those holding them are to be working in clinical fields, they will be encouraged to concentrate on basic science as well.

With respect to budgetary arrangements, too, Dr. Paynter's recommendations are being followed, in that the dental faculty has undertaken responsibility for supporting part of the research in the basic science departments of the university.

As far as organization is concerned, Dr. Neilson reported that it had been impossible to force the same concepts on members of the staff of all the basic science departments of the University, and the faculty is therefore proceeding along different lines in different departments. In some departments, the "project method" of teaching is followed but an attempt has been made to modify it somewhat so that the students will receive some laboratory instruction first, followed by collaboration on a project.

The Faculty has been allotted the sum of \$50,000 to set up its own library, which will be separate from that of the Medical School and will naturally concentrate on the dental literature. An amount of \$3,600 per year will be provided for the purchase of books and periodicals.

With respect to space, Dr. Neilson reported that present plans call for 57,000 sq. ft. immediately adjacent to the Medical School and the Winnipeg General Hospital. In this building, 6,300 sq. ft. has been allotted to research, of which 2,500 sq. ft. will be devoted to an animal penthouse. In addition, there is almost 5,700 sq. ft. of unfinished space which may or may not be devoted to the expansion of research facilities, depending on future requirements.

In closing his remarks, Dr. Neilson thanked the Committee for inviting him to attend its sessions, and said that he would welcome the opportunity of playing host to the Committee if at some future date it could hold its meeting in Winnipeg.

13. General Discussion of Use of Grant Funds

Dr. Marshall attended the meeting briefly and several members took advantage of this opportunity to ask for clarification of Council procedures with respect to the use of grant funds.

Dr. Neilson inquired about the possibility of transferring funds awarded in support of an investigation from one facet of the research to another. Dr. Marshall said that while an application for funds may outline certain uses for any funds which may be received, there is no rule of Council which would prevent a grantee from using the funds somewhat differently than first proposed as long as he remained within the same general framework of the approved proposal. If a grantee finds, for instance, that it would be in the best interests of his research to apply funds originally intended for technical assistance to the purchase of a piece of equipment unexpectedly necessary to the investigation, it would be in order for him to do so, although he might notify the Awards Office of his intention so that the Committee would have an opportunity to comment on the transfer if it wished to do so. Dr. Marshall pointed out that in the final analysis, the results obtained with the grant funds provided will be the criterion on which the work is judged, and the Council does not feel it desirable to hamper the grantee by insisting on the use of all funds as originally planned before the project is actually started.

The one exception to this is the use of grant funds for travel. In each such case, the grantee must specifically request permission to use funds in this way. Dr. Marshall pointed out that Council provides a general research grant to University Presidents for support of research as they see fit. In time, it is hoped that this grant might be partially used to take care of travel by university staff members within Canada and the United States. Travel grants for trips abroad might continue to be the subject of special requests to Council.

Dr. Neilson also asked whether application might be made for funds to be used for the testing of dental materials. Dr. Castaldi agreed that the testing of new products used in operative dentistry would be most desirable. He felt that it is important to find better filling materials, for instance, which will withstand the things they have to withstand in the mouth; he suggested that research in this area should be encouraged by the Committee.

The Chairman replied that such products are normally tested elsewhere and it is unlikely that funds would be provided by the Committee for this purpose. However, applications in this area are not ruled out automatically; each application submitted is judged on its merits in relation to the interests of the Committee and the funds available.

14. Budget for 1960-61

The probable needs of the Committee for funds for 1960-61 were discussed. It was noted that not only the number, but also the calibre, of applications for Fellowships is increasing, and that a greater number of applications for grants can be expected as a result of the programme being developed at the University of Manitoba.

It was MOVED by Dr. Beveridge and SECONDED by Dr. Lussier THAT it be recommended to Council that the budget of the Associate Committee on Dental Research be increased to \$100,000 for 1960-61.

CARRIED

15. President's Remarks

The Chairman welcomed Dr. Steacie who commented briefly on the general financial picture with respect to university support by the Government through the National Research Council. He pointed out that the amount provided for Council's extramural programme had almost doubled in the past few years, and an increase was anticipated for the coming year 1959-60. While it was impossible to make any forecast of future allocations, Dr. Steacie assured the Committee that he saw no likelihood of a reduction in university support through grants and scholarships.

16. Membership of the Committee

The Chairman noted that his own term of appointment on the Committee and those of Dr. Beveridge, Dr. Racey, Dr. Husband and Dr. O'Neil would expire on 31 March, 1959. He asked for nominations for new members, and for the Chairmanship.

It was MOVED by Dr. Lussier and SECONDED by Dr. D'Iorio THAT it be recommended to Council that Dr. K.J. Paynter be appointed to succeed Dr. Copp as Chairman of the Committee.

CARRIED

It was MOVED by Dr. Beveridge and SECONDED by Dr. Scott THAT nominations for Chairman cease.

CARRIED

The names of Dr. L.E. Francis, Dr. I. Kleinberg and Dr. J.W. Neilson were proposed to succeed Dr. A.G. Racey, Dr. C.D. Husband and Dr. R.J. O'Neil on the Committee.

It was MOVED by Dr. Beveridge and SECONDED by Dr. Lussier THAT nominations cease.

CARRIED

It was MOVED by Dr. Beveridge and SECONDED by Dr. D'Iorio THAT Dr. A.C. Gornall of the Department of Pathological Chemistry of the University of Toronto be appointed to replace Dr. Beveridge.

CARRIED

It was MOVED by Dr. Macdonald and SECONDED by Dr. D'Iorio THAT Dr. L.F. Belanger of the Department of Histology of the University of Ottawa be appointed to replace Dr. Copp.

CARRIED

The Secretary was, accordingly, instructed to submit a recommendation to Council that Dr. L.E. Francis, Dr. I. Kleinberg, Dr. J.W. Neilson, Dr. A.C. Gornall and Dr. L.F. Belanger be appointed to the Committee for a three-year term, and that Dr. K.J. Paynter be appointed Chairman for the duration of his term of appointment.

17. Request for Travel Grant

The Chairman informed the Committee that Dr. L.F. Belanger had requested permission to use \$150 of his Term Grant DT-52 to defray the cost of travelling to Washington University, Seattle, to present a paper.

This request was approved.

18. New Journal of Dental Research

Dr. Macdonald informed the Committee that the Pergamon Press of London and New York had recently undertaken the publication of a new journal devoted to dental research, "Archives of Oral Biology".

He noted that Dr. Copp, Dr. Leblond and Dr. Nikiforuk have accepted membership on the Editorial Board of this new journal, the first issue of which is scheduled to appear in April or May of this year.

The meeting adjourned at 2:50 p.m.

Appendix A

ANNUAL REPORT TO

THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL, CANADA

SUBJECT: (a) Synthesis and secretion of protein in saliva.
(b) Secretion of bicarbonate ions in saliva.

AUTHOR: A.S.V. Burgen

LABORATORY: Department of Physiology, McGill University.

GRANT NO.: D.A. 61.

During the current year studies on total protein secretion and in iodine binding by salivary proteins have been completed and are being prepared for publication. The most important new findings are that by paper chromatography the organically bound iodine has been found to be present mainly as 3-iodotyrosine, with smaller amounts of 3:5 diiodotyrosine. Up to the present no evidence for thyroxine or triiodothyronine has been found. Detailed analysis of the iodine incorporation process has shown that less than 5 μ g of iodinated protein is stored per gram of gland. This confirms previous findings that the P_1 (duct) protein is not appreciably stored. The iodine in the gland is concentrated by the outer face of the duct cells and is incorporated into proteins at the inner cell face and then discharged into the duct lumen.

Iodine incorporation occurs into at least three proteins found in saliva, but at present little progress has been made in improving the separation of these proteins.

Perchlorate and thiocyanate both reduce total and protein bound iodine to the same extent.

A start has been made on studying amino acid incorporation into the salivary proteins.

Good progress has been made with the study of bicarbonate secretion. The most important finding has been that most of the bicarbonate of the saliva is secreted by the salivary ducts and that only a minor part is derived from the acinar secretion or from gland metabolism. Bicarbonate concentration in the saliva is largely independent of the plasma concentration except when the plasma bicarbonate falls very low when it may become the limiting factor in bicarbonate secretion. Acetazoleamide is without effect on bicarbonate secretion suggesting that the secretion is of the bicarbonate ion rather than trapping of diffused CO_2 .

Mrs. A. Wechsler is at present writing an M.Sc. thesis on bicarbonate secretion and we hope to prepare the work for publication subsequently.

I am at present preparing a monograph on "The physiology of salivary secretion" with Professor N. Emmelin of Lund, Sweden.

Appendix B

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

January 1959

Subject: Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements.

Grantee: Dr. C.P. Leblond

Project No.: DA 23

Amount of grant: \$3,850.00

SUMMARY OF WORK

In the course of this year, emphasis has been placed on the entry of labelled amino acids into the proteins of growing dentin and enamel. The tritium (H^3) label is being used in view of the high radioautographic resolution obtained with this isotope. So far glycine- H^3 , methionine- H^3 and leucine- H^3 have been investigated.

The best substance to examine the formation of dentin was found to be glycine- H^3 . Soon after injection of this substance, a strong radioautographic reaction appears over what is believed to be the Golgi zone of the odontoblasts. Within a few hours, the reaction is no longer present in the odontoblasts but appears in predentin. These results are interpreted to mean that the collagen of dentin is elaborated within the odontoblasts and deposited to the outside as the collagen of predentin.

The best substance for the study of enamel formation proved to be methionine- H^3 . Again, this substance appears to be synthesized into a protein in the Golgi zone of the ameloblasts and is then deposited to the outside as preenamel.

This work is presented in detail in the enclosed report.

RADIOAUTOGRAPHIC INVESTIGATION OF THE UPTAKEOF LABELLED METHIONINE BY THE DENTIN ANDENAMEL MATRIX OF GROWING TEETH

Irene Karpishka, C.P. Leblond and J. Carneiro

Department of Anatomy, McGill University

Montreal, Canada

Radioautography throws light on the role played by cells in processes of secretion. The first recorded instance was observed with bicarbonate- C^{14} (Greulich and Leblond, 1953) and glucose- C^{14} (Kumamoto, 1956). Examination of the sublingual gland by radioautography soon after injection of either substance, showed the label in the apex of the acinar cells, but later, it was in the mucus visible in the lumen of the excretory ducts. ^{Later,} Examination of growing teeth after injection of tritium-labelled glycine revealed that the label was first in the cytoplasmic proteins of odontoblasts and later was deposited as collagen into the predentinal matrix (Carneiro and Leblond, 1959).

It was decided to examine the uptake of the essential amino-acid methionine, in the tissues of the tooth. The ectodermic nature of enamel led us to hope that perhaps this structure, like hair and other ectodermic derivatives, might take up sulfur-containing amino-acids preferentially. Indeed, Bélanger (1956) had already shown an intense uptake of methionine into growing enamel. In this investigation methionine labelled by sulfur 35

B-2

was used. Here, besides such methionine, radio-carbon (C^{14}) and tritium (H^3) labels were also used in this amino-acid.

MATERIALS AND METHODS

Four male littermate white mice, each weighing 10-14 gm, were given a single subcutaneous injection of 10 μ c L-methionine methyl- C^{14} in 0.04 c.c. of saline per gram body weight. The animals were sacrificed 1 hr., 3 hrs., 24 hrs. and 6 days later, following ether anesthesia.

Ten male C_3H mice, weighing 26-34 gm each, were injected, as above, using a dose of 5 μ c per gram body weight methionine-methyl- H^3 in 0.01 c.c. of saline. They were sacrificed in pairs 30 min., 4 hrs., 35 hrs., 7 days and 35 days later.

Twenty male C_3H mice, weighing 14-16 gm each, were injected subcutaneously using 4 μ c DL-methionine- S^{35} in 0.0064 c.c. saline per gram body weight. They were sacrificed in pairs 1 hr., 4 hrs., 35 hrs., 4 days, 7 days, 21 days, 28 days, 35 days and 45 days later, after ether anesthesia.

In another experiment, 20 male 3-day-old black-and-white hooded rats weighing 7-10 gm, were employed. They were injected with 5 μ c DL-methionine- S^{35} in 0.01 c.c. saline per gram body weight and were sacrificed in pairs 30 min., 3 hrs., 8 hrs., 24 hrs., 3 days, 6 days, 14 days, 30 days, 60 days and 90 days later.

The jaws were fixed in alcohol-formalin and decalcified in versene. Part of the slides for radioautography were stained with hematoxylin-eosin; the rest were left unstained.

Slides to be radioautographed were dipped in 2 baths of 1% celloidin in alcohol-ether (1:1), allowed to dry, and--in the dark--covered with melted NTB3 Kodak emulsion (as in the case of the methionine- S^{35} experiment) or Ilford Nuclear Research (gel) Emulsion (as in the case of the methionine-methyl- C^{14} and methionine- H^3) according to the dipping technique. (Messier

B-3

and Leblond, 1957). These slides were stored in dry, light-tight containers kept at 4°C. The exposure time was evaluated by development of test slides. Radioautographs of underexposed, optimal and overexposed tissue sections were obtained (Exposure time 1-6 months). The latent images formed by β radiation energy on emulsion were developed producing a silver grain blackening (of the emulsion) over those areas of tissue containing radioactivity. The tissue sections were then passed through alcohols, cedar oil, and Canada balsam, and permanently mounted with a cover slip.

RESULTS

Enamel Tissues

In young rats, the distribution pattern of the S^{35} label after methionine- S^{35} injection was examined in the incisors and molars 30 minutes after injection. Two intensely radioactive areas were seen. One was a small region of the ameloblasts located next to the nucleus and a few μ 's below the apical surface of the cell (which faces preenamel). This reaction may be over the Golgi zone of the ameloblasts, which is known to be in the same location. The presence of reaction at the same level in all ameloblasts, which are located side by side, produced a band-like reaction which ran next to the nuclei along the cell row (fig. 1 A). A second reactive band, even more intense than the first one, was found over preenamel in immediate contact with the apical surface of the ameloblasts (fig. 1, horizontal arrow).

In the young rats sacrificed 3 hours after injection, the intracellular radioactivity had nearly disappeared, but an intensified band was seen over preenamel. By 8 hours, the radioactive band, having been displaced by newly-formed non-radioactive preenamel, had moved at a slight distance from the cells. At this time the band appeared broader and slightly less intense. This trend was emphasized at the 1-, 3- and 6-day intervals as the band

B -4

spread out over the so-called young and maturing enamel. By the 14th day, the phenomenon could not be followed further as removal of the calcified enamel by decalcification was associated with loss of the matrix.

In adult mice, the early distribution of methionine-S³⁵, -C¹⁴ and -H³ in growing teeth was just the same as described above. The intracellular radioactive area was seen at 30 minutes and 1 hour after injection, particularly clearly with methionine-H³ (fig. 3 A), but not at later time intervals (fig. 2, A). The preenamel reaction band was seen at 30 minutes and 1 hour (fig. 3, horizontal arrow), but was maximal by 3 or 4 hours, while later a broader and less intense band was seen over young and maturing enamel (fig. 2, horizontal arrow).

Dentinal Tissues

In young rats given methionine-S³⁵, radiosulfur was found in the cytoplasm of the odontoblasts and at the edge of predentin at 30 minutes after injection, but the two bands were weak and usually not distinguishable (except when artefactually separated as at the top left in figure 1). At 3 hours, the cellular reaction had decreased and the predentinal band became more distinct, while by 8 hours this band was seen over dentin proper. The distance between this band reaction and the odontoblasts increased with time, but the distance from the dentino-enamel junction was not altered; nor was the intensity changed even 2 months after injection.

The same results were also true for the incisors of adult mice injected with methionine-C¹⁴ or -H³. Thus, with methionine-H³, rather weak bands were seen at 30 minutes over odontoblasts (fig. 3, 0) and predentin (fig. 3, oblique arrow). The odontoblast reaction band soon disappeared, while the predentinal band intensified and was later seen over dentin (fig. 4). In contrast no specific reaction was seen over the odontoblasts, predentin or dentin in the molars of adult mice.

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DISCUSSION

The uptake of methionine label followed the same pattern in the incisors of young rats and adult mice and also in the molars of young rats, that is to say in all rapidly growing teeth. No uptake of methionine label was seen in the molars of adult mice. Hence the radioautographic observations of the uptake of labelled methionine were associated with tooth growth, that is, with formation of new enamel and dentinal matrix.

It is known that both matrices contain proteins. In enamel there is a very insoluble, little known fibrous protein, while in dentin the protein material is almost exclusively composed of collagen (Stack, 1955).

Various views have been held regarding the role of methionine. Following the injection of large doses of this substance into young rats, Du Vigneaud et al (1956) observed that the methyl group of this amino acid was labile, passing into choline and other substances, and even into carbon dioxide. If this intermediary metabolism phenomenon was as pronounced as believed by these authors, then it would be expected that methionine labelled in the methyl group with C^{14} or H^3 would produce a different radioautographic picture from that obtained with sulfur-labelled methionine. However, in the tooth no differences were obtained with the three types of methionine (figs. 1-4). Unpublished radioautographic results obtained with a variety of tissues showed us that the three labels of methionine are always distributed in exactly the same manner. It is, therefore, felt that the small, physiological doses of methionine which we administered were not subjected to demethylation to a significant degree. Thus, under physiological conditions, the methionine molecule would behave as a whole. Indeed, much evidence has accumulated that methionine is taken up as such into newly-formed proteins (Tarver, 1954; Leblond et al, 1957). Hence the sites of deposition of the label reveal the sites of protein synthesis. It is conclu-

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ded that the radioautographic observations reported above help us visualize the formation of the matrical proteins in growing dentin and enamel.

In dentinal tissues, the uptake of methionine is rather low. At the early interval, the picture was somewhat hazy, but in areas where the odontoblasts were artefactually separated from predentin, it was possible to see that weak bands were present in both odontoblasts and predentin (top of figs. 1 and 3). The disappearance of the odontoblast band between 30 minutes and 3 hours, that is at the time when labelled methionine disappeared from the blood, and the increase in predentinal band intensity led us to conclude that the material appearing in the cells is the precursor of the labelled component of predentin. This interpretation was strengthened by a similar, but far more evident sequence of pictures after injection of tritium-labelled glycine. With this amino-acid, there was little doubt that the strongly radioactive material observed in the odontoblasts at 30 minutes after injection was the precursor of the radioactive material appearing in predentin at 4 hours (Carneiro and Leblond, 1959). The intensity of the reaction was about 20 times as strong with glycine as with methionine, while the content of glycine in collagen is 28 times that of methionine (Bowes and Kenten, 1948). Hence, it was concluded that with both precursors we had indeed visualized the elaboration of collagen by the odontoblasts and its eventual deposition into predentin. Incidentally, with time the reaction appeared to be indefinitely stable, since no changes in the location or intensity of the reaction band was seen (fig. 4) - an observation in line with the stability of collagen in general (Neuberger and Slack, 1953).

In enamel tissues, the observations reported in the present paper allow a precise visualization of the formation of enamel protein. While at the 30 minute interval, reactions were observed in a Golgi-like zone of the ameloblasts and in preenamel, the cellular reaction promptly disappeared and the preenamel reaction intensified. This was taken to mean that a protein is elaborated in the Golgi zone of the cell and released to the outside as

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preenamel. Whether the newly formed protein made up Tomes' processes or the intermediate material could not be clearly established. With time, the matrical reaction band becomes broader and less intense. It seems that this phenomenon may be accounted for by the "dilution" due to the considerable addition of minerals.

SUMMARY

Radioautographic observations in young rats and adult mice, making use of methionine labelled either with S^{35} , C^{14} or H^3 reveal that formation of both dentinal collagen and enamel protein occur in two steps:

1. In the odontoblasts, a collagen or collagen precursor appears within the cytoplasm and is then released as the collagen of predentin.
2. In the ameloblasts, protein material appears in the cytoplasm and is released as enamel protein into preenamel.

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LEGEND

- Radioautographs of unstained incisors taken from rats and mice after radiomethionine injection.

Figs. 1 and 3 indicate S^{35} and H^3 localization at 30 mins. and 1 hr. following administration to a 3-day old rat, and an adult mouse.

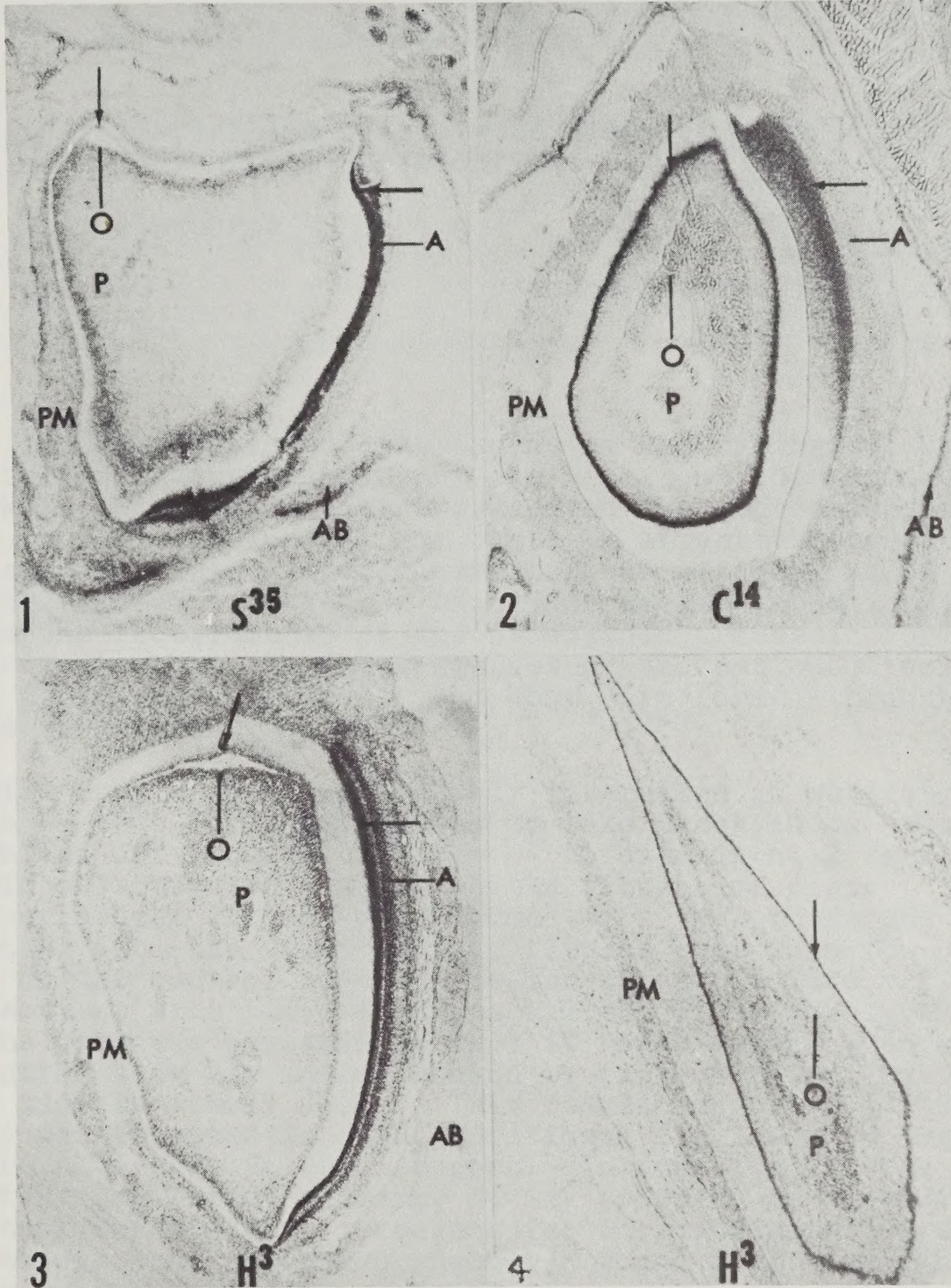
Exposure 5 mo., and 7 mo., X 50.

The intense reactions indicated by horizontal arrows are in pre-enamel. The ameloblasts (A) also contain large amounts of tracer. A similar but weaker pattern is observed over predentin (vertical arrows) and odontoblasts (O). Alveolar bone (AB) also shows a distribution of isotopes in periosteum and osteoid. Pulp and periodontal membrane are slightly radioactive.

Figs. 2 and 4 show C^{14} and H^3 distribution in mice 6 days and 7 days after injection.

Exposure 1 mo., and 4 mo. X 50.

The preenamel radioautograph (horizontal arrow) is less intense because of the spreading of the radioactive band throughout the entire layer, and the enamel reaction is weak. In the dentin the former predental bands (vertical arrow) are separated from the odontoblasts (O) by a thickness of dentin and predentin deposited since the administration of the isotopes. The alveolar band (AB) reaction has also moved into the mature bone, away from the periosteum. Odontoblasts (O), pulp (P), periodontal membrane (PM) and ameloblasts (A) are all weakly radioactive.



Appendix C

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January, 1959

Subject: An investigation of the mechanisms of maintenance and of repair of the periodontium.

Grantee: W.J. Linghorne

Project: D.A. 22

Amount of Grant: \$1,500.00

SUMMARY

In 1958 we continued our studies on the biological processes involved in the maintenance and repair of the periodontium. Prior work in this investigation indicated that the processes operating in the periodontium are those in effect throughout the skeletal system. This finding permitted the utilization of other parts of the skeletal system. This year we are investigating the nature and the requirements of the osteogenic process. Former studies indicate that the three tissues of the periodontium, i.e. cementum, periodontal membrane, and bone, are formed through the osteogenic pathway in repair.

This year, we are particularly interested in subjecting our hypothesis -- that for osteogenesis to take place in an area it must be walled off from soft tissue -- to experimental test. This is the main objective of a study on the fibulae of dogs. In this study, experimental lesions were formed of a type where the cells proliferating into the wound on both fibulae come from the same source. On the control side the lesion was not walled off, while on the treatment side the lesion was walled off by a polyethylene tube. The tube was prepared so that it would expand when necessary. On the control side the lesion healed by fibrous union, on the treatment side by a bony union. This result supported our hypothesis. This study was repeated in eight additional fibulae in 4 dogs. The results are not ready for this report. In addition, if the results of this study are fairly uniform, it may prove to be a valuable research tool for studying osteogenesis; in particular for studying the factors that determine whether a wound heals through the osteogenic or the fibrogenic pathway.

Studies on regeneration of the periodontium indicate that osteogenesis is more difficult to induce in wounds that are open to the surface during the healing period than in wounds that can be closed. Methods for creating the conditions for osteogenesis in wounds that can be closed are unsuitable for open wounds such as the experimental lesions of the periodontium. Therefore a number of the operations were exploratory and will not be dealt with in this report. Four studies are being carried out to study the use of certain procedures suggested by our work on osteogenesis,

and to test their effectiveness in inducing regeneration in experimental lesions. It was found that certain procedures were effective in certain types of periodontal lesions but ineffective on others. We are continuing our work on this problem.

Preliminary studies are being carried out to determine the suitability of a method for studying the nature of the stimuli for cellular proliferation in wound healing.

In 1958, two papers were published in the J.C.D.A. A paper on our work was given to the Annual Meeting of the Dental Society of the State of New York, also another was presented at the 1958 Annual Meeting of the American Academy of Periodontology.

PROGRESS REPORT

Earlier work in this investigation indicates that regeneration of lost periodontal structure may occur in two ways which we have termed physiological and primary regeneration. These terms have been described in earlier reports. Our work this year is mainly to explore further the nature of the primary regenerative process.

Primary regeneration may be defined as that occurring through the organization of a blood clot. Many attempts have been made both experimentally and clinically to utilize this method to induce regeneration of periodontal structure lost through periodontal disease. Various types of therapy have been tried but basically the procedure has been to remove the pocket epithelium and to form a blood clot between the denuded root and the detached gingiva. Results so far have been disappointing. Only very occasionally has worthwhile regeneration been induced. Many reasons for the lack of success have been advanced.

Our experimental findings indicate that the periodontal tissues, i.e. bone, periodontal membrane and cementum, are repaired through the osteogenic pathway. Also, if an osteogenic process is induced, the differentiation of the three periodontal tissues will be automatically provided for and will take place. It may well be that one reason for failure to induce regeneration is that in the vast majority of cases the blood clot formed between the gingiva and the denuded root is organized by fibrous tissue. In most other organs of the body a blood clot is organized by fibrous tissue. That brings up the problem, what are the factors involved in determining an osteogenic rather than a fibrous organization of a clot?

From our experimental findings on this problem, the following hypothesis has evolved. "For osteogenesis, the area to become bone must be walled off from soft tissue. This does not appear to be necessary when autogenous grafts are used and endosteal cells survive".

The following studies using the fibula and the periodontium of dogs were mainly designed for the following objectives:

1. To subject the "walling off" hypothesis to further experimental test.
2. To develop methods of effectively "walling off" periodontal lesions so that restoration of structure and function may occur.
3. To explore further the nature of the primary regenerative process.

STUDY A

Hypothesis

If the hypothesis is valid, for a bony union to occur, the reduction and immobilization of a fracture must effectively wall off the area. If the area is not walled off, healing will result in a fibrous union. The following study was planned to test this hypothesis.

Procedure

In both fibulae of the dog a gap of 3 mm. was produced by the removal of both bone and periosteum. On the left, control, side nothing further was done other than closing the wound. On the right, treatment, side, the graft was bridged by a polyethylene tube. The tube was slipped over the cut ends of the bone to a distance of approximately 4 mm. The tube was of greater diameter than the bone by about 2 times, but was slit longitudinally and folded in to fit the bone. Care was taken to fill the tube with blood. The soft tissue was sutured to prevent the tube from opening. The tube was split because of failures in previous work, possibly through constriction of the periosteum if the tube was too small or failure to wall off the area in the gap from soft tissue if the tube were too big.

Results

A bony union is the criterion that an osteogenic organization of the blood clot has occurred; a fibrous union, a fibrous organization. A photograph of a radiograph taken after healing was completed is enclosed, showing a fibrous union on the control side and bony union on the treatment leg. This procedure was carried out in five dogs to test uniformity of the result. The results are not ready for this report.

STUDIES ON THE PERIODONTIUM

Walling off the proliferating cells in periodontal lesions is much more difficult than with lesions that can be closed. The experimental lesions of the periodontium are open to the surface during the healing period. Inert materials, such as polyethylene used in the fibula, are unsuitable for open lesions as they act as a wick preventing healing. This problem of walling off in open wounds may be complex, therefore at this stage many of the operations are exploratory rather than designed to test the adequacy of a procedure, or the validity of a concept. Where possible the operations are grouped according to a basic procedure. Asterisks indicate variations in the procedures. Exploratory operations that do not fit into the groups are not listed in the Table.

STUDY B

Hypothesis

The basic concept in this study is "walling off" the proliferating cells by replacing the blood clot with solid material covering the bony walls from which cells proliferate. Of course, the solid material must provide at least some of the functions of the blood clot. A type of zinc oxide eugenol cement appears to be reasonably suitable. In 1957, this procedure was tested in three walled intrabony pocket type of lesions with controls healing through the organization of a blood clot. Such lesions are "walled off". In "walled off" lesions of this type regeneration by this method was equal, if not more than, through the organization of a blood clot. In the following study this procedure was tested in periodontal lesions with a base and only one wall. Such lesions are not "walled off". The cases in Study C were used as controls.

Procedure

The two large upper and lower molars were used in this study. A gingival labial flap was made. The labial plate was removed, exposing from 7 mm. to 14 mm. of tooth. The inter-radicular bone was removed exposing the roots in the bifurcation

as far as possible without piercing the lingual plate. The roots were scaled to remove any soft tissues. The space created by the removal of the bone and periosteum was filled, not over filled, with cement and the flap was sutured. After a suitable healing period a gingival flap was again made, exposing the bone and the amount of regeneration noted. The gingival flap was again sutured. The various steps in the procedure were recorded by photographs.

Two variations are included in this study: (1) the cement was wedged into the bifurcation to ascertain the ability of the proliferating cells to push out the cement under such conditions*, and, (2) a large window was cut in the flap over the cement so that the flap would slough away. This was done to study the role of the flap in the healing process**.

Results

The results are summarized in Table I. The amount of regeneration was graded: 4 plus, most regeneration, to 1 plus, little or no regeneration. 3 plus and 2 plus are intermediate grades. Some of the animals were examined at various intervals during healing and the presence or absence of the cement was recorded. The healing time recorded was the interval between the production of the lesions and when the gingival flap was made to ascertain the amount of regeneration. Photographs are enclosed to illustrate grades 4 plus and 3 plus.

It is too early to draw definite conclusions, but our tentative interpretation of the findings may illustrate the problems we are working on.

1. Periodontal lesions may be of two types, (1) where there is a considerable bone surface exposed when the gingiva is retracted and the main source of the cells for regeneration is from the bone, and (2) where there is little bone surface and the main source of the cells is from the periosteum. In type 1, worthwhile regeneration may be induced by means of a suitable solid material. The cement we are using seems reasonably satisfactory. We hope to study regeneration by this method further; the conditions under which it may be used effectively, possible dangers, etc. For example, this year we expect to study this method in lesions with a bony base only. We anticipate additional problems to its use in 3 and 2 walled lesions.
2. This finding supports the "walling off" hypothesis.
3. Just placing the cement in the lesion is not enough. If it is wedged in, regeneration will not occur. On the other hand, if the cement is used in such a way that it becomes loose, little regeneration will occur. We are investigating

a method of holding the cement firmly against the cells, but at the same time permitting the proliferating cells to push the cement out. We hope to report the principle in the next report.

STUDY C

Study C was used as a control for Study B. Identical lesions were made as in Study B but allowed to heal through the organization of a blood clot. To prevent the "walling off" of the lesion by the periosteum on the gingival flap, a window was cut in the flap over the bone lesion, in an attempt to create a 2-walled pocket. As shown on the chart some regeneration did occur. This result surprised us as when the flap was accidentally perforated in our studies on cuspid teeth no regeneration occurred. One tentative conclusion from this and a later finding in epithelialized pockets (Study D) suggested that when considering regeneration through just the organization of a blood clot, lesions may again be classified (a) those pockets where there was a small bone surface and the main source of the cells for regeneration is from the periosteum; (b) those pockets with a considerable bone surface where the main source of the cells is from the bone. The amount of regeneration through the formation and organization of a blood clot is affected by the above factors, there being much more in the (b) than in the (a) type. The increased regeneration in the (b) type may be the result of differences in the rate of cellular proliferation, but we suspect that the presence of more devitalized bone on the larger bone surface in its effect on differentiation is the more important factor. Further understanding of the sequence of events in the regenerative process and their inter-relationship is required to explain this observation.

STUDY D

In this study lesions were prepared in the labial of the canines similar to those used in Studies 1 and 2 (supra bony pockets). As indicated in the Table, attempts to secure regeneration in this type of lesion, by means of the cement were entirely unsuccessful. This type of pocket presents only a minimum amount of bone surface. Also the bony surface in this type of lesion in the dog is largely compact bone. Our studies indicate that the main source of the cells for regeneration in this type of lesion is from the periosteum at the margins of the wound. The use of the cement appears to be ineffective in promoting regeneration in this type of lesion.

STUDY E

After the gingival flap was made to ascertain the amount of regeneration in Studies B and C, the lesions were allowed to heal. At this time the lesions were comparable to clinical periodontal lesions. Any pockets were epithelialized. However, gingival recession was the usual result of lost periodontal tissue rather than pocket formation. We have been attempting to regenerate both the lost periodontium and the lost gingiva. The four cases reported in the Table show little regeneration, although the result in one case showed promise. On the basis of this result, we have changed the procedure using the same lesions. It is too early to report definite conclusions.

A number of exploratory operations were performed that do not fit into the above studies. These are not recorded in the Table. Altogether, 76 operations were performed on dogs which were recorded by 271 photographs and 8 radiographs.

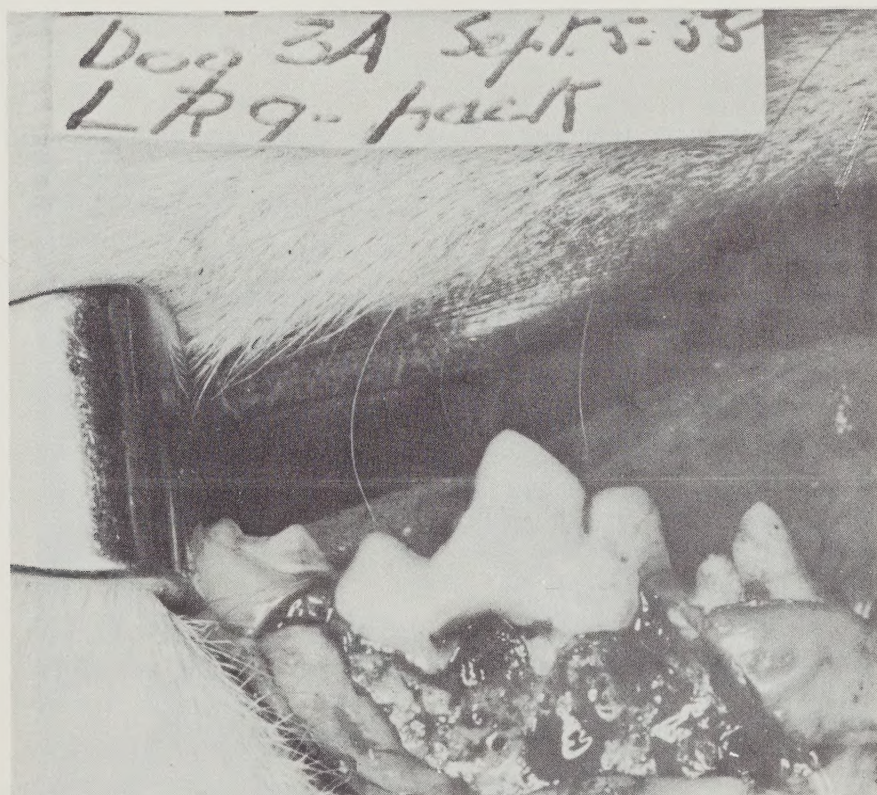
New bone formation may be divided into four steps: cellular proliferation, differentiation, the formation of osteoid tissue, and its calcification. For the repair of breaks in the continuity of bone or of soft tissues cellular proliferation is required. Cellular proliferation must be initiated, maintained until continuity is established and then stopped. What is the mechanism by which cellular proliferation is initiated and maintained in repair? Preliminary studies suggested by our work with polyethylene tubes indicate the possibility that this research tool may be useful in investigating the problem. We propose to add this study to our investigation of the repair of skeletal tissues. Among other things further light on the normal mechanisms for initiating normal cellular proliferations in repair may be helpful in investigating abnormal proliferation.

REGENERATION OF BONE

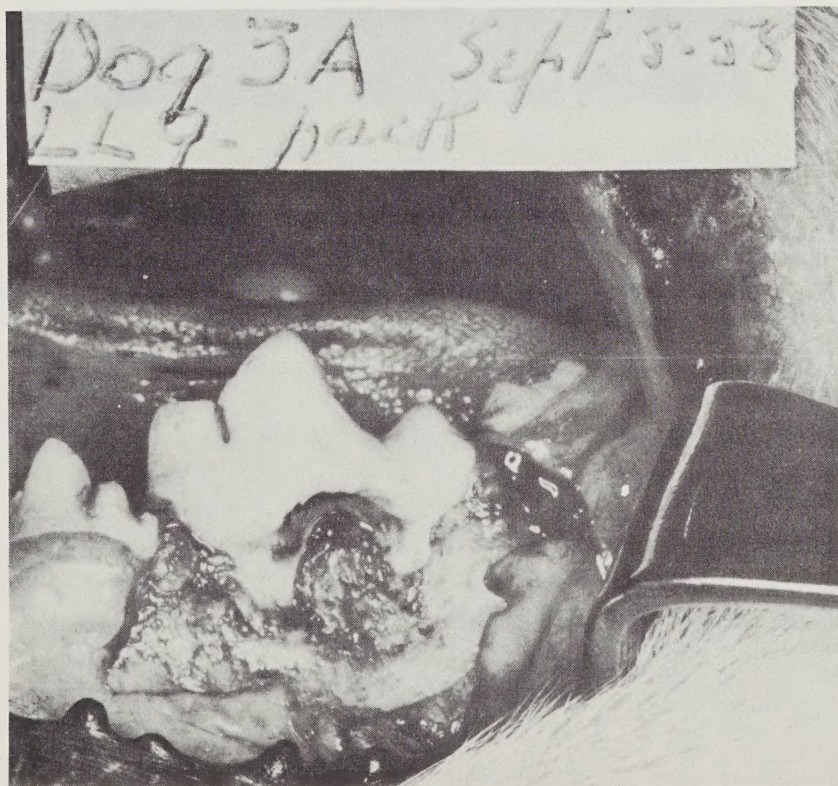
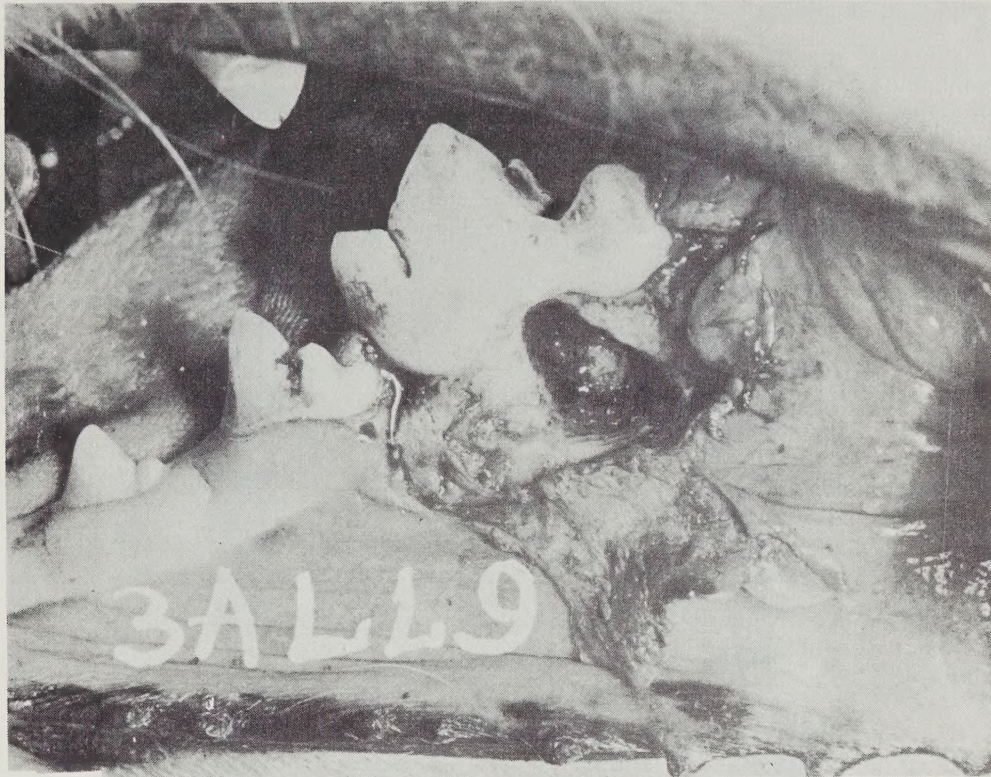
Study	Tooth designation	Post-operative time, in days	Disappearance of applied cement, in days	Degree of regeneration
B	1-A	55	27	+++
B	2-A	97	21	+++
B	3-A	177		+++
B	5-A	Not completed		
B	6-A	"		
B	7-A	"		
B*	2-A	62	62	+
B**	3-A	156	28	+++
B**	5-A	83		+++
C	2-A	97		++
C	3-A	55		++
C	5-A	83		++
C	6-A	Not completed		
C*	5-A	"		
C*	7-A	"		
D	1-A	27	27	+
D	1-A	27	27	+
D	3-A	34	34	+
E	2-A	38		++
E	2-A	41		+
E	5-A	Not completed	20	
E	5-A	"	Replaced in 20 days	

C - 8

B* = Cement was wedged
 B** = Window cut in gingival flap over cement
 C* = No window in gingival flap

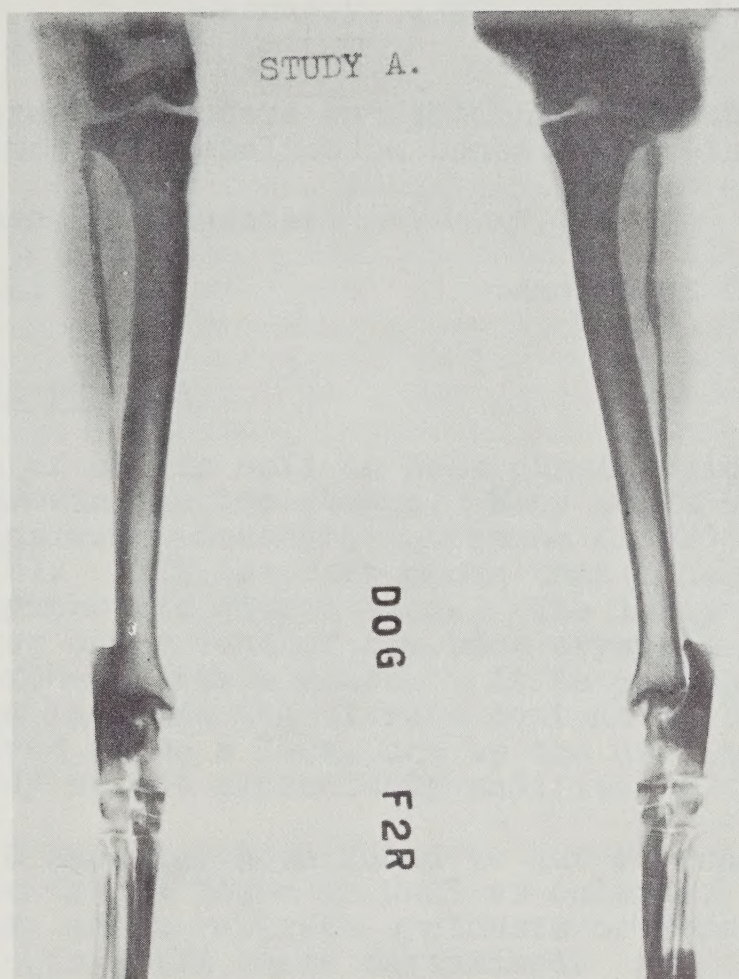


4 plus



3 plus

C-11



Appendix D

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January, 1959

Subject: Hormonal factors and their relationships of citrate metabolism in bones and teeth.

Grantee: Jean-Paul Lussier

Project No.: D.A. 70 Amount of Grant: \$6,360.00

INTRODUCTION AND PURPOSE

The role of citric acid in bone physiological processes is constantly growing in importance. Many works have pointed out its relationships with parathyroid hormone (1)(2)(3) and vitamin D (4)(5)(6)(7)(8). It has been shown that bone contains enzymatic systems which synthesize citric acid. The latter readily exchanges with phosphate or other ions of the bone crystal and is retained until further exchange takes place. It is possible through the action of PTH to increase the citrate content in bone. This effect is believed to be a local one as the utilization of citrate in the whole body is not appreciably modified.

Vitamin D has also been found to act on bone citrate as it releases citrate to the blood as well as calcium. Whether this effect is primary on the citrate synthesis or secondary to some other osteolytic is still to be determined.

It is important to know about this key compound if hormonal factors which act anabolically on bone, like sex hormones, are not related in some way to the mechanisms which regulate its synthesis and utilization. Estrogens are known to promote spongy bone formation in man, mouse, rat, and laying hen. In the latter species they also provoke hypercalcemia. The present work has been undertaken to shed further light on this aspect of bone metabolism.

Fluoroacetate being a potent inhibitor of citrogenase, its administration rapidly produces an accumulation of citric acid in the tissues. It should be possible by this means to follow the incorporation of radioacetate in bone citrate and to study the influence of other factors upon it like parathormone or vitamin D.

This type of study, however, cannot be conducted without a follow up of the citrate metabolism in the whole body as illustrated in Schemas I and II.

The rate of utilization and excretion of citrate must be known as accurately as possible because quantitatively the changes which can occur in the whole body are by far much larger and more rapid than in the skeleton. It has been determined (9) that bone incorporates at most about 5% of an injected dose of radiocitric acid.

It is assumed that any change which may happen in bone citrate can be the result of influences acting in situ or indirectly through alterations of the metabolic activities of the soft tissues. It thus becomes imperative that all experimental animals injected with radioactive citric acid have their respiratory gases and urine analysed for their content in radioactive compounds. At this stage of our experiments, indicative data have been collected concerning the respiratory CO₂ and urine analysis. Hard tissues analysis have been delayed in order to improve the method previously used when the whole skeleton was used. It is intended now to depend only on two representative bones (tibia and femur) in which zones of different metabolic activity can be more accurately delimited (epiphysis, metaphysis, diaphysis).

EXPERIMENTAL PROCEDURE

Part I - Effect of sex hormones on citrate metabolism

1- Animals treated with sex hormones

Rats of both sexes of the Long-Evans Strain were oophorectomized at 30 days of age. Half of the males received four injections of 3 mg. testosterone propionate in corn oil every three days for two weeks. Half of the females received four injections of 2 mg. estradiol benzoate in corn oil at the same frequency. The other halves were injected with corn oil as controls.

Each animal was injected with two milliliters of a solution containing 2 mg. citric acid tagged in the carboxylic groups with carbon 14. The radioactivity of the injection is about one million counts per minute as determined with a thin window GM tube. The animal is placed in a metabolic cage, the respiratory CO₂ and the urine are collected as previously described (9).

All animals at the time of sacrifice were anesthetized with ether; blood samples were collected through heart puncture. Tibias and femurs were removed, cleaned of muscles and kept at -20°C.

2- Bone analysis

A weighed amount of bone chips is introduced in a modified Lang-Farber flask, followed by 20 ml. of TCA 20%. The CO₂ evolved

is displaced by means of suction pump towards an absorbent flask containing sodium hydroxide 10%. The L.F. flask is heated up to boiling temperature to expel all the CO_2 from the solution. CO_2 is precipitated with BaCl_2 and the precipitate is washed, dried and weighed. The radioactivity is determined on an aliquot of the precipitate. This first step is necessary to remove CO_2 adsorbed in the bone crystal as carbonate.

The bone solution is filtered and the filtrate is returned in the L.F. flask. KBr , H_2SO_4 and KMnO_4 are added in proportions and concentrations according to Bauer et al. (10). The CO_2 evolved from the oxidation of citric acid is collected and the radioactivity recorded as above.

This method has not been found accurate enough when the bone samples were under 100 mg. in weight. A new analytical scheme was developed and summarized in Schema III.

A weighed amount of bone chips (50-100 mg.) is introduced in a combustion tube and connected to a Van Slyke manometric apparatus. 5 ml. of H_2SO_4 20% are introduced after the gas has been expelled out of the apparatus. The tube is heated and boiling is maintained for six minutes which appeared to be a safe period for complete removal of CO_2 from the solution (Table I).

TABLE I

Time of Heating	Amount of C (microgm.)	
<u>Min.</u>	<u>Present</u>	<u>Founds *</u>
2	75	45
4	75	72
4	75	77
6	75	70
8	75	75

* mean of 2 determinations

The maximum vacuum is obtained by lowering the mercury bulb to 50 line of the gas burette. The connection is closed between the tube and the apparatus. CO_2 is then measured according to the regular technique. After the readings are made, the solution remaining in the Van Slyke apparatus is recuperated and kept for radioactive measurements (Sol. A). Table II illustrates some of the results obtained with this technique.

TABLE II

Van Slyke determination Bone CO₂

Test Solution

Sol. NaHCO ₃	Present	Founds *	% Error
171.9 microg/ml	90	89.2	1%
195 " "	100	98	-2%
195 " "	100	102	+2%
195 " "	100	103.5	+3.5%

* mean of 5 determinations

Bone chips	CO ₂ % *
Epiphysis	2.06
Metaphysis	2.14
Diaphysis	4.15

* mean of 3 determinations

The sulfuric solution (Solution B) is centrifuged, decanted and diluted to 10 ml. Aliquots are used for citric acid determination according to Bauer et al. (10). The rest is used for chromatographic analysis. At this stage we are working to improve the technique in order to remove from solution B the excess of SO₄ and other ions which may interfere with the migration of citrate and other organic ions on the filter paper. Removal by barium is not advisable as most of the radioactive materials remain absorbed on the precipitate.

Extraction by organic solvents (amyl acetate and propyl alcohol) is effective, but their evaporation under infra red light leads to partial destruction of organic acids. Evaporation under

vacuum is too long to be practical. Purification on ion exchange resin is the next step to be worked out. The last part of the technique is the same as the one used for urine analysis.

3- Chromatographic analysis of urine

Urine is washed out from the bottom of the metabolic cage, diluted to volume, divided in equal portions, dried under vacuum and extracted with different solvents: HCl 1N, NaOH 1N, and alcohol 95%. Aliquots of these extracts are then chromatographed on Whatman paper No. 1 using the following mixtures.

- a) methylcellosolve: 80, NH_4OH : 5, H_2O : 15, for acid extract (11)
- b) butanol: 90, H_2O : 5, formic acid: 5, for alkaline extract (12)
- c) alcohol 95%: 80, NH_4OH : 10, H_2O : 10, for alcoholic extract (13)

One dimension descending chromatography has been used in every case. The $R_f \times 100$ values of citric and lactic acid are shown in Table III.

Table III

$R_f \times 100$ values

I		II		III
<u>Solvents</u> - BuOH - HCO_2H		<u>MiCellosolve</u> - $\text{NH}_4\text{OH}-\text{H}_2\text{O}$		<u>EtoH</u> - $\text{NH}_4\text{OH}-\text{H}_2\text{O}$
	95 : 5		80: 5: 15	8: 1: 1
citric acid	42		16	10
lactic acid	70		66	64

Chromatography of radioactive citric acid present in urine (Chrom.I)

Radioactive citric acid when chromatographed in methylcellosolve, ammonia and water does not give a definite spot but a smear of 12 cm. long. An impurity not identified (probably a by-product of the synthesis, as it does not appear in all batches from the manufacturers) shows a peak at 20 cm. When an equal amount of radiocitric acid is added to an aliquot of urine (Chrom.II) from a normal rat (40) the same characteristics are found plus another peak at 25 cm. This peak was found to coincide with the peak of lactic acid.

Urine voided at different times was assayed chromatographically and the distribution of radioactivity between citric and lactic acids estimated. Citric acid is excreted shortly after the injection, and lactic acid a little later at the end of the first hour or so. At this point it can be stated that the amount of citric acid injected which escapes the metabolic pathway is very small, about 1% of the amount injected. The portion catabolised into lactic acid and excreted as such in the normal animals is about the same.

RESULTS

Radioactivity of the respiratory CO_2 and urine have been determined in animals treated with sex hormones.

Effect of testosterone

The content of radioactive CO_2 has been determined in three animals for four and a half hours following the injection of radio-citric acid. The values are shown in Graph. I and compare to the normal curve (continuous line). In two animals the rate of production was markedly decreased, in the other it was half way between the latter and the normal controls. One castrated animal showed a rate of production of radioactive CO_2 lower when compared to normal. It has been reported by Hayano (11) and Gordan (12) that testosterone inhibits aerobic respiration in kidney, liver, muscle and brain. The findings about the testosterone are in agreement with these facts. The catabolism of citric acid is delayed and consequently the rate of appearance of radioactive respiratory CO_2 is decreased.

Although the values of radioactivity in urine of the three animals treated with testosterone show wide variations, there is an indication that lactic acid is excreted in larger proportion. Normal gives a citric/lactic=1.0. The testosterone treated gives 0.06, 0.6, and 0.9. Values from the orchidectomized control are comparable to normal.

Effect of estradiol

Only two animals have been investigated on, one oophorectomized and treated with estradiol and one oophorectomized control. Radioactive respiratory CO_2 is lowered in both case. It may be that ovariectomy has an inhibitory action in the cycle as the amount of radioactive CO_2 is somewhat lower than the normals after a five hour observation period, but the experience must be repeated before any clear indication is made reliable.

The chromatograms of urine samples do not disclose any difference from the normal.

These preliminary findings show that citric and lactic acid excretion may be affected by the treatment with testosterone but not with estradiol. However the changes in the respiratory elimination of radioactive carbon dioxide suggest that they will have to be taken into account when the results on bone will have to be interpreted.

PART II - Effect of parathyroid hormone on animals under the influence of fluoroacetate

Twenty-three animals (60-80 gms) were used to determine the levels of citrate metabolism at different doses of fluoroacetate and to evaluate the influence of parathyroid hormone thereon.

Here is a list of the different groups: No. of animals

a) Normal controls	2
b) Fluoroacetate injected 10 mg/kg body weight	5
c) " " 5 mg/kg " "	4
d) " " 2.5 mg/kg " "	2
e) " " 5 mg/kg plus PTH	5
f) PTH " controls	5

1- The normals were injected radio-citric-acid

(one million cpm). The radioactivity recovered in respiratory CO₂ one hour after the injection was 35.2% and 42%. One sample of urine contained 1.8%, the other was lost.

2- Fluoroacetate 10 mg/kg

From the five animals injected with 10 mg/kg of fluoroacetate, only one survived until the end of experiment. Citric acid was administered five hours after fluoroacetate. The survivor did not void any urine and the bladder was found empty. The respiratory CO₂ retained 3.5% of the injected radioactivity.

3- Fluoracetate 5 mg/kg

In this group all the animals stood the treatment long enough to reach the end of the experiment. Values are 7.0, 4.2 and 6.3% for the respiratory CO₂ and 0.7, 0.9 and 2.1% for urine. One animal was injected with fluoroacetate two instead of five hours before citric acid. 6.7% and 0.3% respectively for CO₂ and urine.

4- Fluoroacetate 2.5 mg/kg

At this dose, the amount of radioactivity recovered in respiratory CO_2 is about one third less than normals $\text{CO}_2 = 25$ and 23.6%; urine: 2.1%.

5- PTH was assayed in three different ways in animals treated with F-Acetate

- a) 15 units have been given at the rate of 5 units every two hours. Fluoroacetate (5 mg/kg) was injected two hours after the last PTH injection and citric acid five hours after F-Acetate.

Values are 1.2, 1.8 and 6.9% for respiratory CO_2

Values are 5.5% in one case, insignificant in the second and undetermined in the third.

- b) 50 units in a single injection 10 hours before F-Acetate.

Values are 7.8 and 11.8% in CO_2 .

Values have not been determined in urine.

- c) Simultaneous injections of 50 units and 50 mg/kg of F-Acetate. Time of sacrifice: five hours after. CO_2 values are 1.7 and 1.3%.

There was no urine voided and the bladder was empty.

6- The effect of PTH has been controlled in normal animals

- a) with 15 units in three injections.
 CO_2 value is 33.%

- b) with 50 units after 10 hours.
 CO_2 values are 38.2 and 24.6%

- c) with 50 units after 5 hours.
 CO_2 values are 20% and 13.3%

COMMENTS AND CONCLUSIONS

The data collected up to now is still very fragmentary. It shows nevertheless that the rate of CO_2 excretion is a fair indicator of the utilization of citric acid. It is true that the rate of disappearance from the blood is an indispensable value to account for the rate of incorporation or exchange with bone constituents. The rate of CO_2 production accounts for the rate of

utilization which is also important in any appreciation of metabolic survey, especially in the case of rapidly utilized compounds.

Any findings that can be anticipated from bone analysis will be considered in comparison with the results already obtained. The male hormone is responsible for a certain inhibitory action in the rate of utilization of citric acid. About the female sex hormone such an action has not been put into evidence although ovariectomy may be per se a factor of variation. The parathyroid hormone in one instance has been shown to depress the rate of utilization. This fact has to be repeated and analysed carefully as it is not in the line of previous results observed in our laboratory before.

The maximum dose of fluoroacetate compatible with a good survival rate is 5.0 mg/kg of body weight.

The simultaneous injection of parathyroid hormone and fluoroacetate is more effective in lowering the utilization of citrate than when fluoroacetate injection follows PTH administration (Graph. III).

Hard tissues analyses are awaiting improvements in the method for purification of citrate solutions.

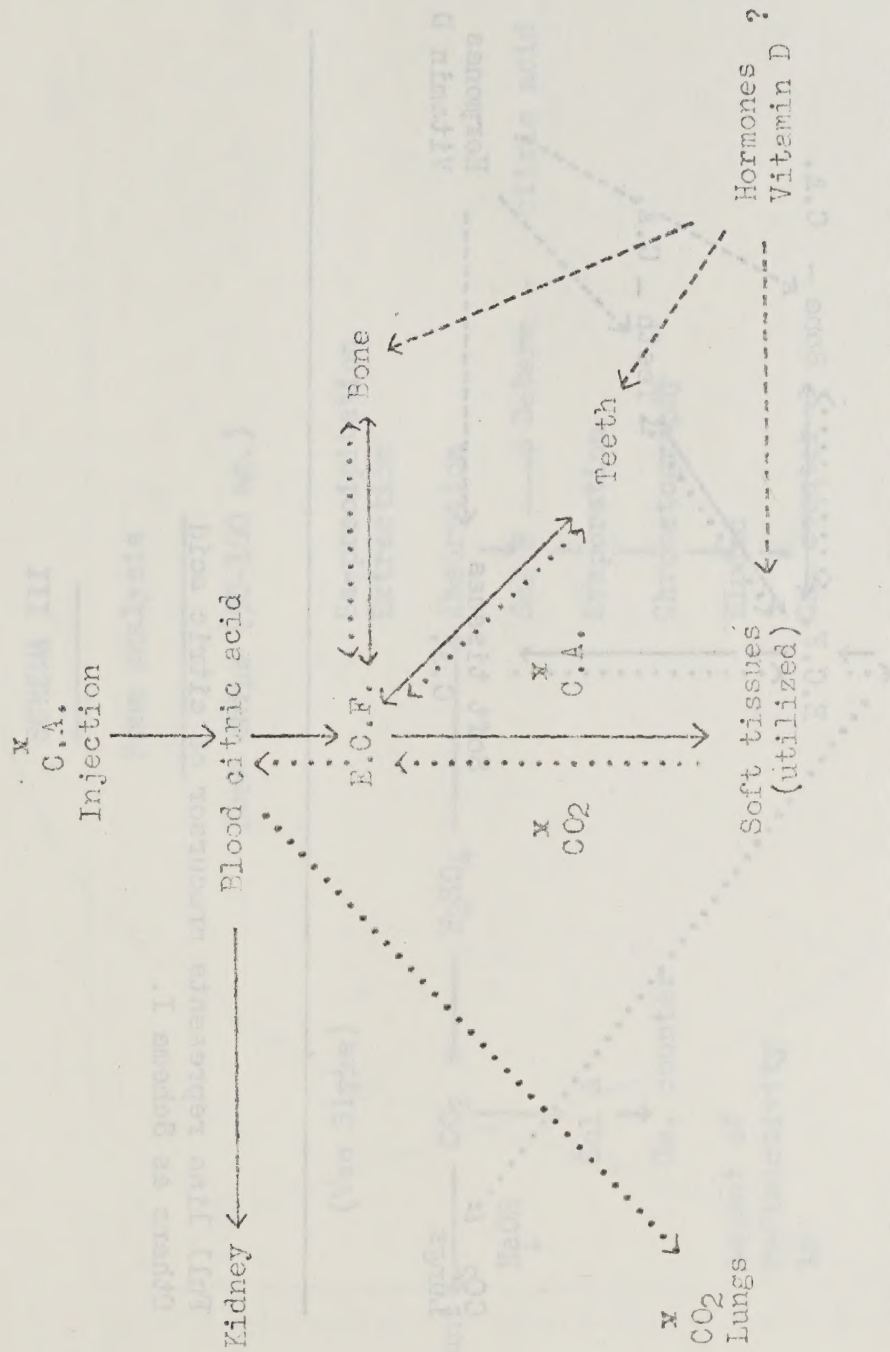
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COMMENTS AND CONCLUSIONS

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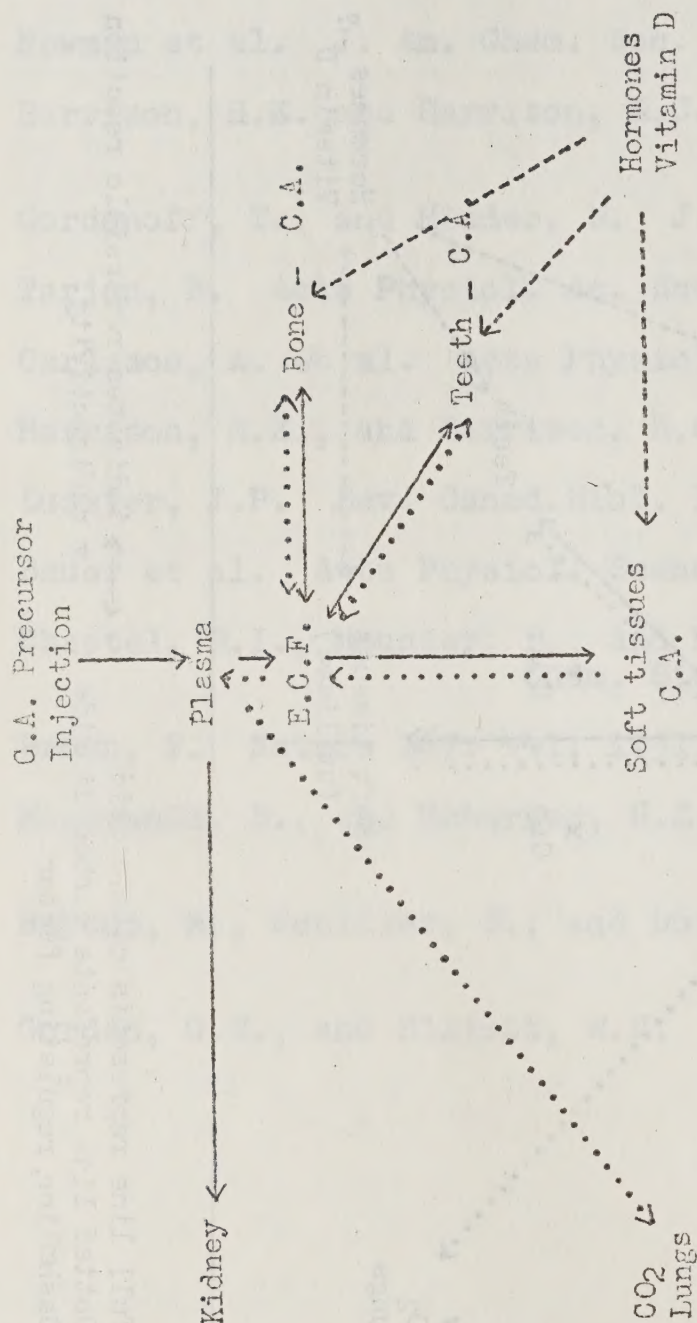
SCHEMA I



Full line represents citric acid
Dotted line represents carbon dioxide
Dashed line, regulating factor

C.A. - citric acid
E.C.F. - Extra cellular fluid

SCHEMA II

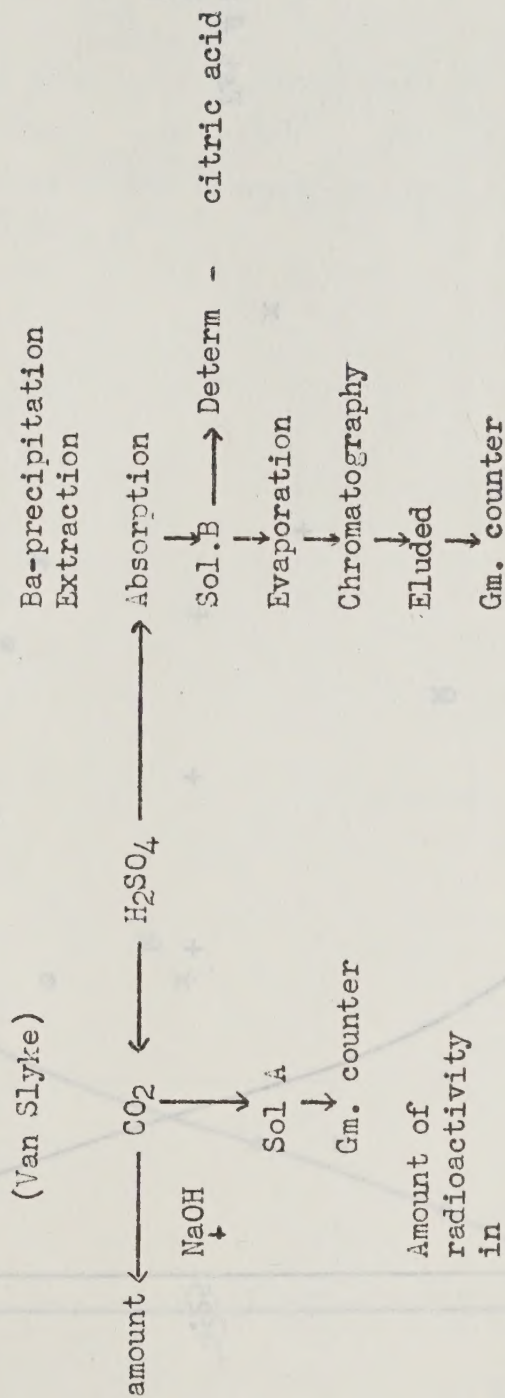


Full line represents precursor of citric acid
Others as Schema I.

SCHEMA III

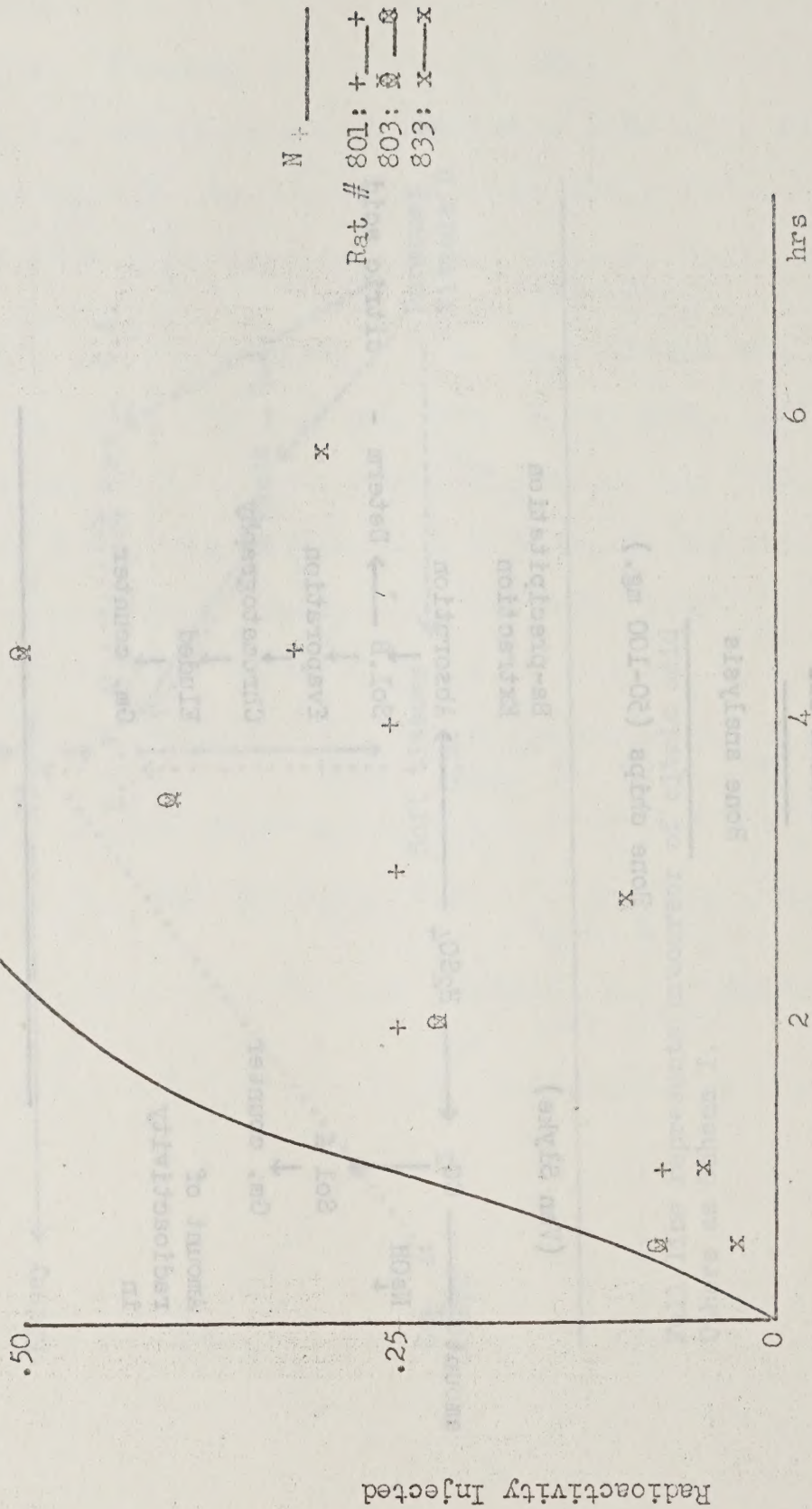
Bone analysis

Bone chips (50-100 mg.)



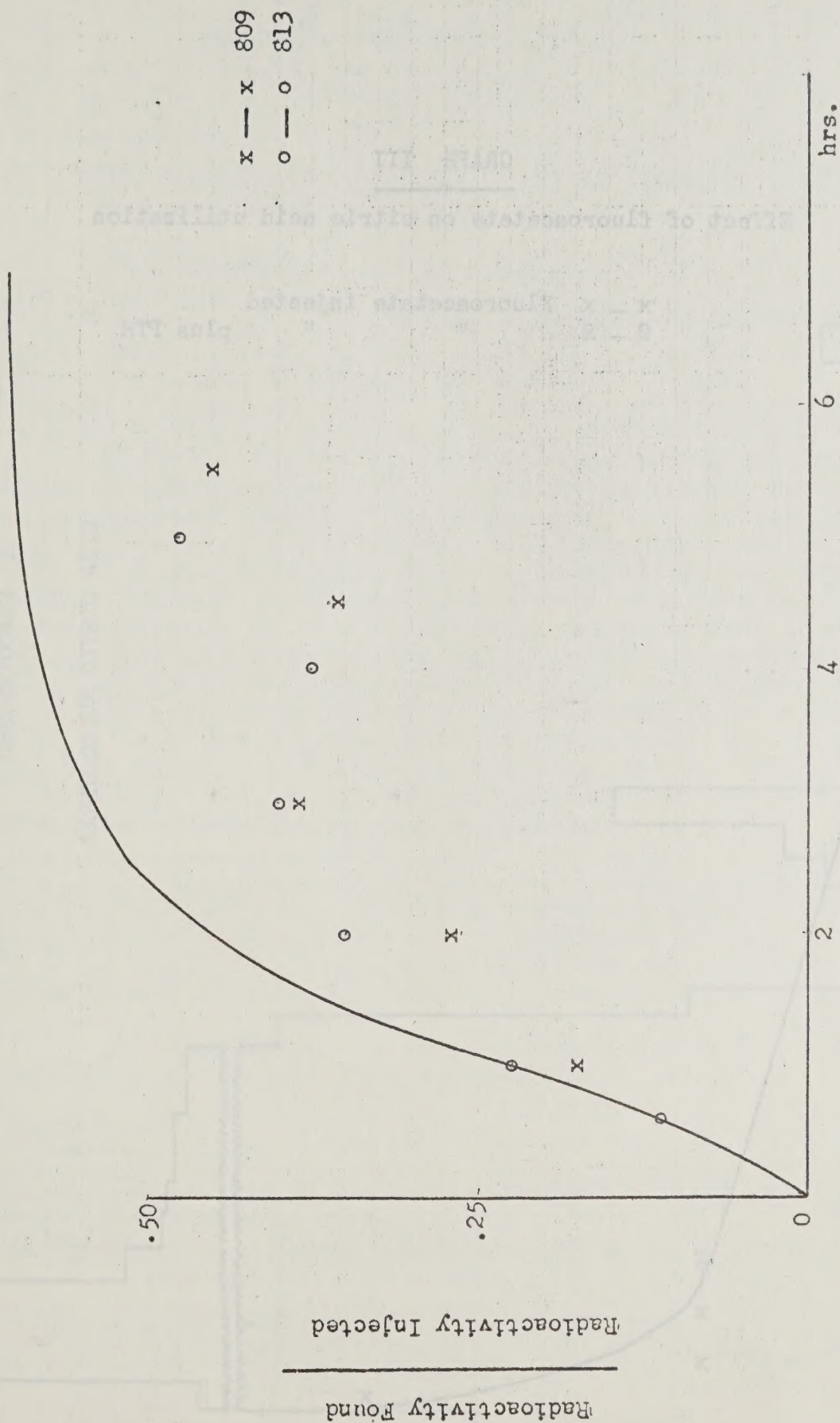
GRAPH I

Effect of testosterone propionate on
the elimination of radioactive CO₂



GRAPH II

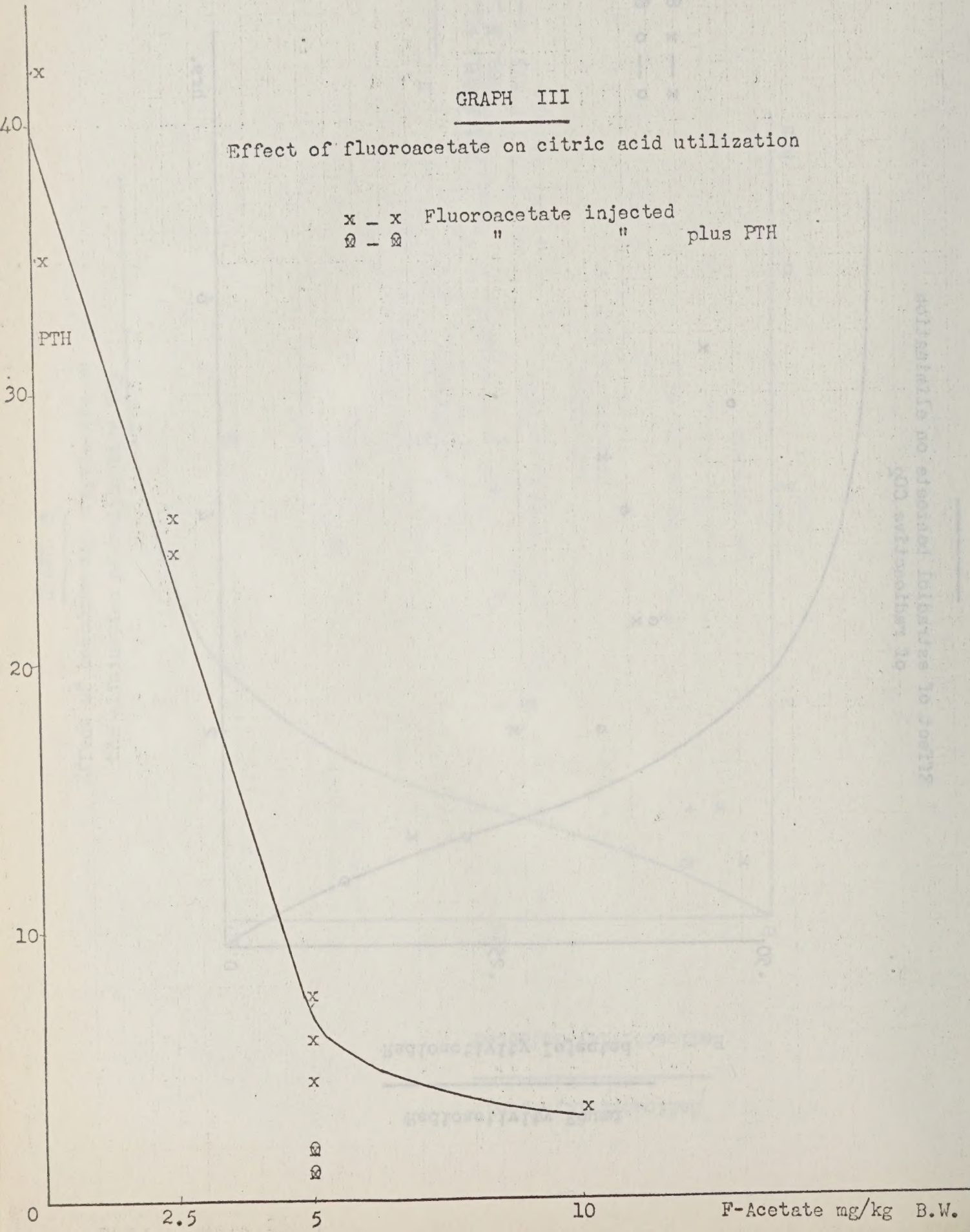
Effect of estradiol benzoate on elimination
of radioactive CO₂



GRAPH III

Effect of fluoroacetate on citric acid utilization

x - x Fluoroacetate injected
 o - o " " plus PTH



REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January, 1959

Subject: The characteristics of an experimental
"aspirin-like" lesion

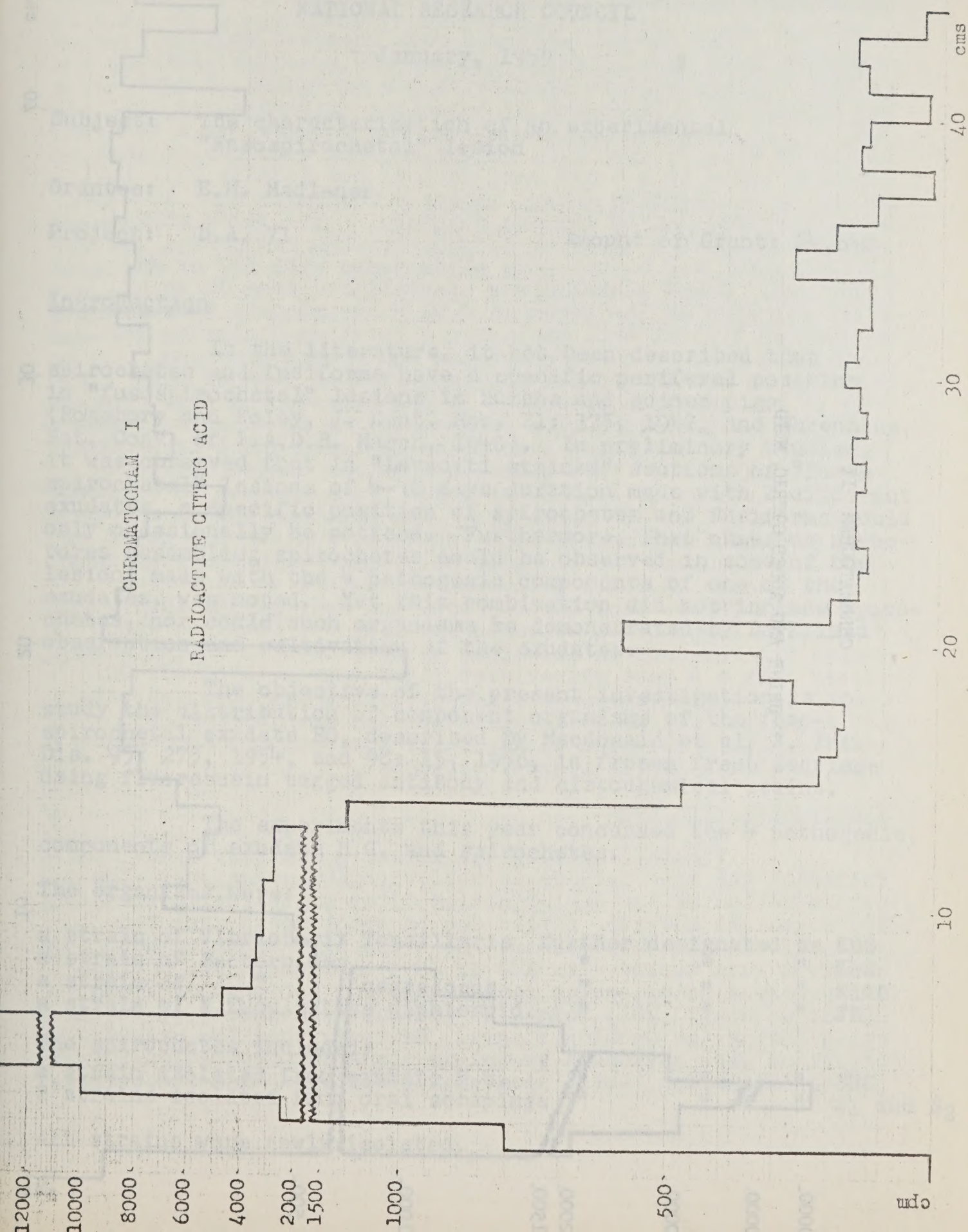
Grantee: E. W. Hallgren

Project: D.A. 72

Introduction

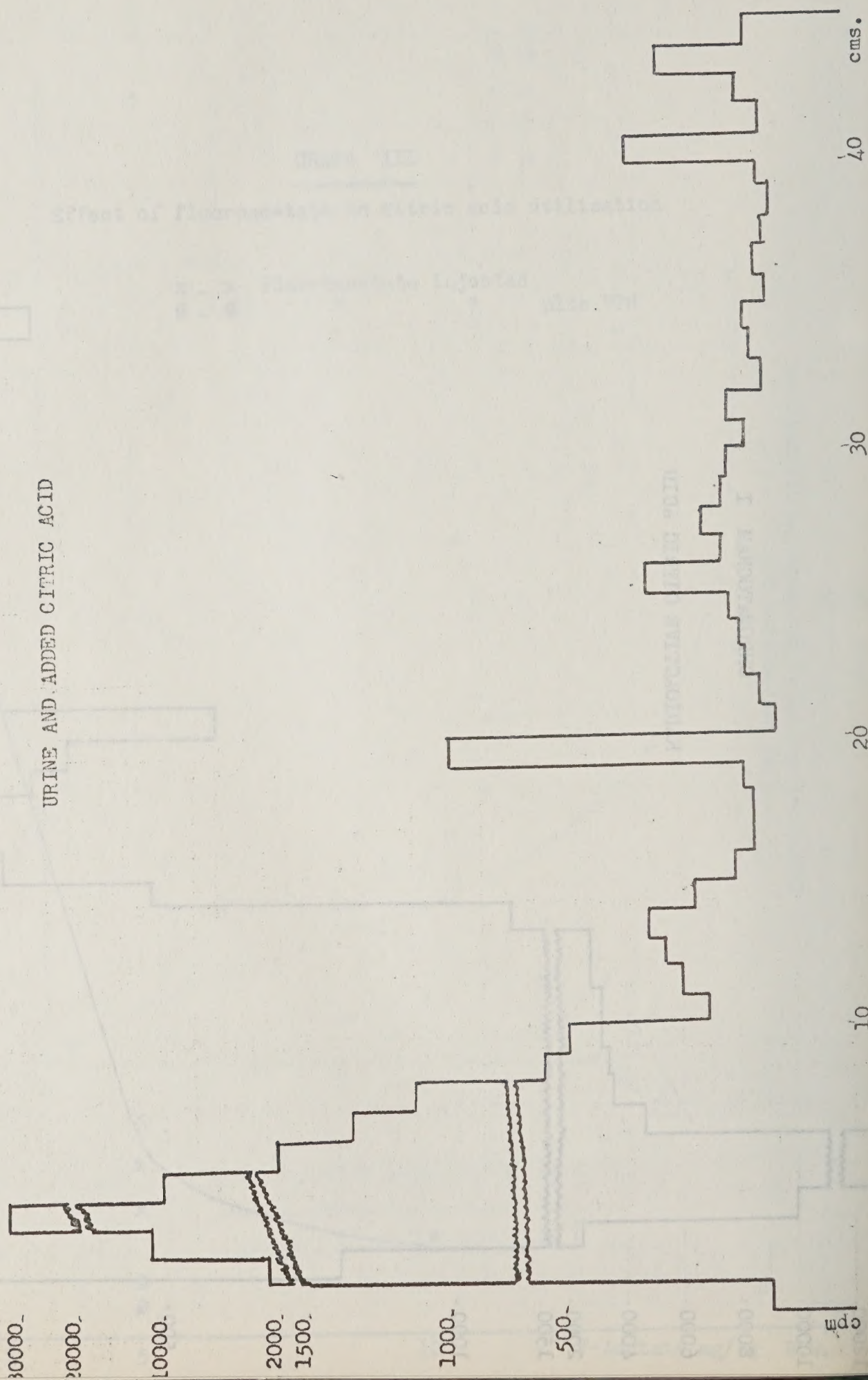
CHROMATOGRAM I

RADIOACTIVE CITRIC ACID



CHROMATOGRAM II

URINE AND ADDED CITRIC ACID



Appendix E

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January, 1959

Subject: The characterization of an experimental
"Fusospirochetal" lesion

Grantee: E.M. Madlener

Project: D.A. 71

Amount of Grant: \$4,640

Introduction

In the literature, it has been described that spirochetes and fusiforms have a specific peripheral position in "fusospirochetal" lesions in humans and guinea pigs (Rosebury and Foley, J. Dent. Res. 21: 375, 1942, and Ehrenhaus, Nat. Conv. of I.A.D.R. March, 1940). In preliminary studies, it was observed that in "Levaditi stained" sections of "fusospirochetal" lesions of 4-40 days duration made with 2 different exudates, a specific position of spirochetes and fusiforms could only occasionally be noticed. Furthermore, that numerous structures resembling spirochetes could be observed in some of the lesions made with the 4 pathogenic components of one of the exudates, was noted. Yet this combination did not include spirochetes, nor could such organisms be demonstrated by Darkfield observation and cultivation of the exudates.

The objective of the present investigation is to study the distribution of component organisms of the fusospirochetal exudate HG, described by Macdonald et al, J. Inf. Dis. 95: 275, 1954, and 98: 15, 1956, in frozen fresh sections using fluorescein tagged antibody and histochemical stains.

The experiments this year concerned the 4 pathogenic components of exudate H.G. and spirochetes.

The organisms were:

a strain of *Vibriothrix Tonsillar* further designated as K80
a strain of *Bacteroides* " " " K92
a strain of " *negrescens* " " " K110
a strain of a facultative diptheroid " " " JR3

The spirochetes included:

a strain isolated from exudate H.G. " " " SHG
2 strains isolated from oral scrapings " " " S1 and S2

All strains were newly isolated.

Experiments with K80

Vaccine and antigens were prepared from organisms from mass cultures (100 cc) in a medium similar to Difco thioglycollate broth with the exception of agar. Two rabbits were immunized by injecting intravenously 0.1, 0.2, 0.5 ml vaccine on consecutive days followed by a rest period of 4 days; then 0.2, 0.5, 1.0 ml on consecutive days followed again by a 4 day rest period, and finally 0.5, 1.0, 2.0 ml on consecutive days. After a rest period of 10 days, serum was obtained by cardiac puncture. The serum showed agglutination to a titer of 1:2560 with K80 organisms. No agglutination was observed in slide tests with K92 or JR3 organisms. Gamma globulin was precipitated from 10 cc of a 1:1 dilution of the serum in 0.01 M bicarbonate buffered saline (pH 7.2) at 4°C, employing a rotating dialyzing bag containing 5 cc of buffered saline and enough ammonium sulphate to obtain a final concentration of 1.6M of this salt in the combined solutions in and outside the bag. The precipitated globulins were redissolved in 2½ cc of buffered saline and the resulting suspension dialyzed until free from ammonium sulphate. This globulin solution showed agglutination to a titer of 1:10160 with K80 organisms and no agglutination with K92 or JR3 organisms. The solution was adjusted with carbonate buffered saline (pH 9) to contain 1 mg of protein per cc and the globulin was tagged with fluorescein isothiocyanate (B.B.L.) following the method as described by Marshal (Proc. Soc. Exptl. Biol. and Med. p. 98, 1958). Finally, a light brown coloured solution was obtained which showed fluorescence in dilutions up to 1:1280 in saline when tested with a 9 watt ultraviolet light source (2560 Å). When tested for agglutination a titer of 1:2540 was observed with K80 organisms and no cross agglutination with K92 or JR3 antigen. This solution is now ready for staining K80 organisms in slides and in tissue sections.

Experiments with K92

Vaccine, antigen and immune serum were made following the same methods as used with K80 organisms. The determination of the agglutination titer of the serum for K92 organisms however, failed due to spontaneous agglutination of the K92 suspensions. No cross agglutination was observed when tested with K80 or JR 3 antigens. New antigen was prepared using saline containing 0.01 per cent of Tween 20 for washing. This seemed to prevent spontaneous agglutination during the washing stages. However, when the washed organisms were suspended in saline alone in preparation for the agglutination tests, spontaneous agglutination reoccurred.

It was found that the pH of the saline suspension of the organisms was 4.8 while the stock saline solutions were at pH 6.2. To investigate if spontaneous agglutination was due to this acidity, a 20 cc culture of K92 was rapidly washed and the pH of the final suspension was determined daily thereafter. It was found that the pH dropped to 4.9 within 7 days; and, at the same time, spontaneous agglutination of the organisms was accelerated. Acid is apparently eluted from the killed organisms in saline suspensions.

When washed K92 cells were suspended in 0.01 M phosphate buffered saline solutions in a pH range from 6.9 to 7.9, it was found that the suspension of pH 7.2 showed the least agglutination. Agglutination tests using buffered saline for serially diluting serum and suspending organisms gave, however, irregular results. Subsequent pH determinations of each tube in the test series showed variation of 0.5, probably due to the large glass surface as compared with the small volume of fluid in the narrow tubes used in the "microagglutination" test. When the buffer was augmented to 0.1 M, spontaneous agglutination was sufficiently prevented to satisfactorily determine the titer of the serum. This titer was determined at 1:2580.

It was felt that the determination of the agglutination titer was a necessary step in order to have a check on the stability of the globulins in the purification and tagging procedures which will now be performed.

Experiments with K110

Introduction

This strain is routinely maintained on blood agar plates cross streaked with staph aureus under anaerobic conditions. It was reported (N.R.C. Senior Fellowship Report, 1957) that this organism was sensitive to hydrogen in non agar containing media and could be maintained in an agar free medium to which was added sheep's blood and growth factor filtered from staph aureus growth in peptone water when incubated in a nitrogen atmosphere. Growth was observed to occur on the glass wall of the culture tubes and on an agar slope which was added in the bottom part of the culture tube.

Experiments

Growth in the above mentioned tubes is light, and mass cultures (100 cc) failed to produce the desired results. As it was noted that better results were often obtained when a glass filter was used instead of a "Seitz" filter for filtering growth factor, it was attempted to introduce growth factor

in low agar (0.05%) and agar free media by inserting a piece of agar cut out from an agar plate next to a heavy growth of staph aureus or JR3 growth. This procedure resulted in a much improved growth of K110 in low agar (0.05%) containing media only. Comparative growth in agar free media could only be obtained when the addition of extra drops of sheep's blood was omitted, and blood plates were used for the process of obtaining and transferring growth factor. From these results it was concluded that the amount of haemoglobin transferred with the cutout piece of blood agar was apparently sufficient for growth of K110, and that addition of extra sheep's blood inhibited growth in agar free media. It was subsequently noted (1) upon reincubation of cultures of heavy turbid growth of K110 (McFarland scale #4) in low agar plus extra sheep's blood containing media, this turbidity disappeared progressively with time, and (2) the rate of disappearance was related to the amount of haemoglobin added. K110 is apparently subject to autolysis. In an attempt to repeat these observations in agar free media, it was found that the amount of growth of K110, (estimated by measuring turbidity), developed during the first incubation period directly related with the amount of haemoglobin added to the media. From these experiments, it was concluded that K110 apparently is subject to autolysis under the direct influence of the amounts of haemoglobin present in the media, and that the process of autolysis was slowed down by the presence of agar.

In an attempt to better control the amount of haemoglobin, a modified "Levinthal medium" was used of the following recipe: a 5:1 mixture of Difco veal infusion broth and citrated sheep's blood is heated to 85°C for 20 minutes. The resulting mixture is centrifuged and the supernate filtered through a "Seitz" filter. The filtrate is added 20% by volume to Difco veal infusion broth. Dextrose, thio-glycollate and Me-blue are added to an amount of 0.5, 0.05, and 0.00005 per cent respectively.

The experiments were repeated in this medium and gave the same results. At that time, it was attempted to employ growth factor as it is recently prepared by Dr. Gratton in the bacteriological laboratory of the Forsyth Dental Infirmary in Boston. Ether extracts of growth of staph aureus and of JR3 were added to the modified "Levinthal" base. The experiments were repeated obtaining the same results. In addition, it was found that the amount of haemoglobin, or a break down product thereof, was sufficient to produce heavy turbid growth (McFarland scale #6) in agar free media.

Using the modified "Levinthal" medium with added ether extract of JR3 cultures in peptone water, heavy mass cultures (100 cc) of K110 could be obtained in agar free media. This result will greatly facilitate the production of antigen and vaccine and determinations of enzymatic activities.

Experiments with Spirochetes

A standard method to maintain strains of oral spirochetes is cultivation in a medium made of "Proske and Sayers base" with addition of 10% ascitic fluid, 0.1% agar and methelene blue 1/500,000 as an oxidation-reduction indicator. The nature of this medium makes preparation of antigens difficult. When strain S₁ was isolated from oral scrapings it was mixed with an organism which greatly promoted growth. Heavy growth of the mixture was obtained after 2 days incubation in a medium made from "Proske and Sayers" base with addition of 0.1% ascitic fluid, 0.05% of agar and Me-blue even when this medium was in the oxidized state when inoculated. Growth of the mixture effectively reduced the medium. Growth of the mixture could also be obtained in "Difco thioglycollate broth" and in agar free Proske and Sayers base without ascitic fluid in 3 serial subcultures made from growth in the above mentioned agar and ascitic fluid containing medium. In further serial subcultures, growth of the spirochetes progressively failed. While the organism, which was a motile rod in ascitic fluid containing media, changed its morphology into ring shaped structures. The organism was a gram positive 2-4 micron long motile rod when grown in ascitic fluid containing media. In media without ascitic fluid, non motile, 2-8 microns long organisms were seen and an equal amount of ring shaped structures 5-8 microns in diameter. Biochemical activity in non ascitic fluid containing media was as follows: Dextrose +, succrose -, mannitol -, indol +, nitrate -. The organism is a facultative aerobe growing on heart infusion plates forming a glossy, low convex, 1-2 mm diam. yellowish colony.

Attempts to obtain a growth factor for spirochetes from filtrates of cultures of this organism grown in ascitic fluid containing, and non ascitic fluid containing media failed.

Assuming that no growth factor was involved in the growth promoting effect of this organism but that this effect was the result of a proper adjustment of the oxidation-reduction potential of the medium, attempts were made to duplicate this potential.

Duplicating the method of potential adjustment used in growing K80 organisms, it was found that the 3 strains of

spirochetes could be maintained in a medium containing Proske and Sayers base, 1 per cent of ascitic fluid, 0.05% thioglycollate, 0.05% agar and Me-blue incubated in a nitrogen atmosphere. Strain S₁ and S₂ were successfully 2 X serially subcultured in the same medium without agar and reduced with nitrogen. Organisms from these cultures could be easily washed. Darkfield observation of the suspensions showed that the organisms were satisfactorily distributed in the saline.

Summary

The objective of the investigation was to study the distribution of organisms in fusospirochetal lesions in guinea pigs using fluorescein tagged antibodies. This year, the experiments concerned four strains of organisms constituting the pathogenic component of an experimental fusospirochetal infection HG (Macdonald, et al) and oral spirochetes. The experiments have progressed as follows. Purified gamma globulin from serum of rabbits immunized with strain K80 of *Vibriothrix Tonsillaris* species was tagged with fluorescein isothiocyanate. This suspension will be used for staining K80 organisms. The determination of the titer to which serum of rabbits immunized with strain K92 of a *Bacteroides* species would agglutinate the homologous organism failed because of the occurrence of spontaneous agglutination. It was found that: (1) this spontaneous agglutination could be prevented by using buffered saline at pH 7.2 for washing, suspending and diluting purposes, (2) the pH range in which spontaneous agglutination is avoided is narrow. The determination of the agglutination titer was a necessary step to have a check on the stability of the agglutinating properties of globulins before the purification and tagging procedures could be undertaken.

Mass cultures of strain K110 of a *Bacteroides negrescence* species in agar free media were unsatisfactory, probably as a result of autolysis of the organisms. It was found (1) the rate of autolysis depended on the amount of haemoglobin present (2) small amount of haemoglobin was needed for growth (3) the presence of agar retarded autolysis. Using these findings, mass cultures could be made in modified "Levinthal" broth to which was added a growth factor developed in a culture of strain JR3 of *Diphtheroid* species. Saline suspensions were made from spirochetes grown in agar free Proske and Sayers media containing 1 per cent ascitic fluid. In darkfield observations, the suspensions looked promising for use as antigens.

Appendix F

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January, 1959

Subject: Studies on the mechanism of gingival changes associated with drug administrations.

Grantee: K.I. Melville

Project: D.A. 66 Amount of Grant: \$4,000.00

Since the last report to the Committee, the complete findings of this investigation over the past three years were brought together in a thesis, submitted by Dr. Lyman Francis and approved for the M.Sc. degree in this Department. A summary of the work to that point has already been sent to the Secretary of the Associate Committee (May 2, 1958). It was clear from these findings that under the influence of dilantin there were characteristic changes in gingival histamine and gingival 5-hydroxytryptamine.

Using the techniques previously described, the work has been continued during the year in conjunction with Dr. Lyman Francis and a part-time technician Mrs. Barbara Levine. In the first group of experiments attempts were made to assess the influence of some antihistamine agents (diphenhydramine, and promethazine) upon the gingival tissue changes induced by diphenylhydantoin (dilantin) in dogs. No definite influence of these antihistamines could however be observed in short-term experiments, and owing to technical difficulties involved in maintaining the animals for long periods on combinations of dilantin and the antihistamines, no definite conclusions can yet be drawn from the data available. On the other hand, we have now also carried out a series of experiments in which the animals have been maintained on a chemical precursor of 5-hydroxytryptamine i.e. one of the enzymes or coenzymes known to be necessary for the formation of 5-hydroxytryptophane and hence for the synthesis of 5-hydroxytryptamine. This material contained in the vitamin-B complex has so far been fed to young ferrets maintained for 8 weeks on oral dilantin, as in our previous experiments. At present it would appear that there is considerably less gingival hyperplasia developing in such animals, than in control dilantin-fed animals. However, the data are still too incomplete to warrant any definite conclusions and the experiments are being continued.

Since it was observed that associated with the effect of dilantin on the gums, there was a loss of serotonin, it was of interest to study the influence of dilantin upon serotonin changes in brain tissue. Some preliminary data on this question in rats would seem to indicate that there is a reduction in brain serotonin concomitant with that of gingival serotonin. Whether or not this is responsible for the central nervous system depressive effects of dilantin cannot yet be stated. This problem is also being studied further.

Since the last report to the Committee, the Committee has been kept advised of the progress of the work. The findings of this investigation over the past three years have been brought together in a thesis submitted by Dr. M. L. Levine and approved for the M. Sc. degree in the Department of Physiology. The thesis is a summary of the work to date and has been accepted for publication in the Journal of the Association of Anatomists (May 2, 1950). The thesis is a clear presentation of the findings that dilantin causes a decrease in the levels of serotonin in the brain and in the gingiva. The thesis also contains a detailed description of the techniques used in the investigation. The work has been continued during the past year in connection with the Dr. Lyman Briggs and a part-time fellowship from the National Science Foundation. In the first group of experiments, the effect of dilantin on the levels of serotonin in the brain and in the gingiva was studied. To assess the influence of some anticonvulsants and some anesthetics (barbiturate, and pentobarbital) upon the gingival levels of serotonin, the influence of these anticonvulsants could however be observed in short-term experiments, and owing to technical difficulties involved in maintaining the animals for long periods on continuous dilantin and the anticonvulsants, no definite conclusions can yet be drawn from the data available. On the other hand, we have now carried out a series of experiments in which the animals have been maintained on a chemical preparation of 5-hydroxytryptamine, i.e. one of the enzymes or coenzymes known to be necessary for the formation of 5-hydroxytryptamine and hence for the synthesis of 5-hydroxytryptamine. This material contained in the vitamin-B complex has so far been found to young rats maintained for 3 weeks on oral dilantin. In our previous experiments, it was evident that dilantin caused a decrease in the levels of serotonin in the brain and in the gingiva. However, such animals, even in control dilantin-free animals, the data are still too incomplete to warrant any definite conclusions and the experiments are being continued.

Appendix G

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January, 1959

Subject: Investigations into the nature of cementum
Grantee: K.J. Paynter
Project: D.A. 64 Amount of Grant: \$2,000

Introduction:

The primary purpose of this study was to follow, histochemically and autoradiographically, the development of the dental tissues in sections of the teeth and jaws of rats. Where demineralization would remove the isotope, the tissues were to be prepared by embedding in plastic without demineralization, and sectioning on a special microtome at six microns. Preliminary exploration had indicated the possibility and potential value of the use of the technique. It was planned to study the isotope distribution in the jaws of young rats following the injection of S^{35} (both as sulphate and as labelled Methionine), Ca^{45} , P^{32} , and H^3 -labelled Thymidine. The isotopes were to be given at a time when as many developmental processes as possible could be studied at the same time in the same section. Fifteen days was selected as the best time for injection, because at this age the first molar has just erupted and come into limited function, the third molar is still in the form of an enamel organ and the crown not yet completed, and the second molar is in an intermediate stage of development when the roots are forming.

During the past year, many of the technical difficulties inevitable in such a study have been ironed out, and considerable progress has been made toward completion of the project.

Procedures:

1. Sixteen 15 day-old rats were injected with 1 m.c./gram of tritium-labelled thymidine, and pairs of animals were killed at intervals of 1, 2, and 3 hours and 1, 2, 4, 7 and 21 days following the injections. The heads of the animals were fixed in formalin, demineralized, and embedded in paraffin for sectioning. Sections were dipped in photographic emulsion (Eastman Kodak NTB #2, Experimental Photographic Emulsion), and exposed for various periods up to 25 days before developing

and staining. Preliminary trials of the technique were successful, but unfortunately, the emulsion used during the actual run of the experiment proved to be from a poor batch of material which became detached from the slides during developing.

Additional sections from the same animals have now been dipped using a new batch of emulsion, and are now being exposed. We expect to have final results of this aspect of the study within the next few weeks.

2. An additional sixteen rats of the same age were injected with S^{35} as sulphate, and these were sacrificed at the same times as those above. After fixation, the jaws of the animals were embedded without demineralization, in plastic (Ward's Bioplastic) for sectioning, and for autoradiography. The problems of preparing such sections have been taken care of, although certain other unforeseen difficulties have considerably delayed progress. We could not find a solvent that would remove the Bioplastic from the sections and not harm the tissues and unfortunately the plastic exposed the photographic emulsion chemically, which so distorted the image that interpretation was impossible. Section staining was also affected adversely and unpredictably by the plastic.

However, some preliminary work with another plastic embedding material which is readily soluble in several laboratory reagents, indicates that its use will remove most of our present difficulties and pave the way for completion of the project, including the proposed studies using Ca^{45} and P^{32} .

3. When we received the batch of S^{35} -labelled Methionine, it was accompanied by a letter from the manufacturer reporting that it was impure. It was thus not suitable for the study planned and we are now awaiting shipment of a new batch.

Rather than waste the impure material, Dr. A.M. Hunt, who is doing the autoradiographic work in this experiment and Dr. Gordon Kikiforuk, collaborated to use it in a trial study combining the techniques of autoradiography and paper chromatography. Their purpose was to ascertain whether these techniques might show not only where in a tooth the radioactivity was deposited following the administration of S^{35} Methionine, but also, in precisely what form it was deposited. Accordingly, the labelled Methionine

was injected into eleven rats--three doses in each animal, at 1, 2, and 5 days after birth. The animals were killed when twenty days old, and one-half the mandible prepared for autoradiography. The rest of the teeth were pooled and separated into the following fractions: soluble and insoluble enamel, organic and inorganic dentin--both soluble and insoluble. The various fractions were dried and tested for radioactivity using a thin end-window geiger counter. It was interesting to find that activity was detected in each of the fractions and in varying degrees, and further that they could find the activity in the spots on a chromatogram following separation by placing NTB 2 photographic plates in apposition to the chromatograms.

Although no interpretation of the results is possible because of the impurity of the material used, nevertheless, the trial has served to establish the value of continuing studies of this type.

When offspring of these females reached 20 days of age, they were anesthetized and the upper right first molar extracted from each animal. The teeth were fixed in formalin, and stored in alcohol for further processing. The animals were then maintained on a complete synthetic diet containing 60 per cent sucrose as carbohydrate, for a period of 180 days, sacrificed--and their various records recorded.

The number of females in each group and the total number of surviving young on which observations were made are shown in Table 1.

TABLE 1

Total number of female rats in each group, the number which bore litters, the total number of young in the litters, and the number of young which survived to 20 days, 180 of which were spent on a synthetic diet containing 60 per cent sucrose as carbohydrate.

Dietary Regime	No. of Mothers	No. of Litters	Total No. of Young	Surviving
Chow	14	14	112	43
Comp. Synthetic	15	13	104	9
Pl. Water	14	11	102	12
High POU	21	19	89	7
Vit. A Def.	21	14	122	4
Totals	85	70	511	75

Appendix H

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH NATIONAL RESEARCH COUNCIL

January, 1959

Subject: The relation of nutrition to morphology and caries-susceptibility in the teeth of rats

Grantee: K.J. Paynter and A.M. Hunt

Project: D.A. 72

Amount of Grant: \$2,700

Materials and Methods:

Young adult female albino rats (Carworth strain) were placed in one of five groups depending on the diet fed the animals during pregnancy and lactation. The group arrangement was similar to that previously described, (Paynter and Grainger, Jour. Canad. Dent. Assoc. 22(9): 519, 1956) except that only one high phosphate group was studied.

When offspring of these females reached 21 days of age, they were anaesthetized and the upper right first molar extracted from each animal. The teeth were fixed in formalin, and stored in alcohol for further processing. The animals were then maintained on a complete synthetic diet containing 60 per cent sucrose as carbohydrate, for a period of 180 days, sacrificed--and their caries scores recorded.

The number of females in each group and the total number of surviving young on which observations have been made are shown in Table 1.

TABLE 1.

Total number of female rats in each group, the number which bore litters, the total number of young in the litters, and the number of young which survived to 200 days, 180 of which were spent on a synthetic diet containing 60 per cent sucrose as carbohydrate.

Dietary Regime	No. of Mothers	No. of Litters	Total No. Animals in Litters	Survivors
Chow	14	14	119	43
Comp. Synthetic	15	13	104	9
Fl. Water	14	11	105	12
High PO ₄	21	16	85	7
Vit. A. Def.	21	16	139	6
Totals	85	70	552	77

Photographs were made of the extracted molars from the survivors under conditions of standard enlargement. Pictures of each tooth included the lingual view of the crown, the occlusal view, and a view of the cut surface of the crown after hemi-section mesio-distally and cervico-occlusally. Measurements of the teeth made from the photographs included the greatest mesio-distal diameter of the crown, the mesio-distal diameter of the crown at the cervix, the bucco-lingual diameter and the height of the crown, and the depth and the angle of the sides of the mesial fissure as seen both in the lingual view and in the sectioned tooth.

Using these methods, it is possible to compare morphological details of the teeth with the subsequent caries experience in the same animals.

Results:

We have not yet had time to do a statistical analysis of the data, but some observations have been made on the basis of a simple graphical analysis as follows:

1. The pattern of alterations in size and shape of rat molars produced by feeding various types of diets during tooth development (Paynter and Grainger, J. Canad. Dent. Assoc. 22(9): 519, 1956) has been confirmed.
2. It was possible to relate differences in caries-susceptibility in animals with differences in size and shape of their teeth. In the control (chow) group, the number of cavities in an animal was related directly to tooth size, particularly in terms of the mesio-distal diameter of the crown at the cervix; i.e. it appeared that whether animals got many cavities was dependent on their teeth being larger than average. The more susceptible animals tended to have less bell-shaped teeth, i.e. the total mesio-distal diameter of the crown and the mesio-distal diameter of the cervix tended to be more nearly similar. In addition, the ratio of total mesio-distal diameter of the crown to bucco-lingual diameter in the teeth of the more susceptible animals tended to be higher.
3. Differences in caries susceptibility were observed between experimental groups. Caries scores in these groups were also related to tooth morphology, although not necessarily in the same manner as in the control group. For example, the teeth in all the experimental

groups were smaller than average (the high PO_4^{4-} ones were smallest), yet the caries experience of the groups varied markedly. Fluoride animals got least caries, the Vitamin A deficient group had a similar experience to the controls, and the high PO_4^{4-} group were by far the most caries-susceptible.

Discussion:

The large difference between the total number of animals born in the groups and the number which survived the experimental period (Table 1) may be accounted for in two ways: first, as is the case with most so-called complete synthetic diets, many of the animals showed some manifestations of non-specific nutritional deficiency before the termination of the experimental period. This was particularly so with the animals raised initially on one of the deficient diets. And second, during the summer of 1958, we experienced a very severe epidemic of what was thought to be a paratyphoid infection in our rat colony which killed a large number of animals.

The photographic techniques now employed were worked out in detail during a study begun in 1957 and completed during the past summer, of the teeth of a caries-susceptible and a caries-immune strain of rats from laboratories of the Harvard School of Dental Medicine in Boston, Mass. The report of this study has been accepted by the Journal of Dental Research for publication and is expected to appear shortly. (Grainger, R.M., K.J. Paynter, J.H. Shaw, J. Dent. Res. In press, 1959). It was found in this work that the teeth of the susceptible animals (a strain which had been developed by breeding on the basis of caries-susceptibility) were smaller than average when compared with the teeth from immune animals, and that the sides of the mesial fissure as seen in the sectioned teeth, were more nearly parallel.

The relationship between the fact that variations in tooth morphology and in caries susceptibility can be inherited, as well as derived from developmental environment, cannot be explained as yet.

Summary and Future Plans:

It is well established that caries-susceptibility as well as tooth morphology can be affected by varying the diet of an animal during the time the teeth are developing, and that the morphological alterations are related--either directly or indirectly--to the caries experience in the animals. The precise nature of the present data, i.e. by

being able to compare the details of morphology of a single tooth from an animal to the subsequent caries score in the same animal, should enable us to move on to a closer understanding of the nature of this relationship.

It has been interesting to observe in the Harvard study mentioned above, that the genetically transmitted susceptibility is also accompanied by characteristic alterations in tooth morphology.

Additional animals will now be added to those groups in the present study which were most seriously depleted.

Future experiments in this series are being designed to learn more of the causative factors in alterations in tooth morphology, i.e. to find out whether the alterations are caused by specific dietary factors or are the result of non-specific nutritional stress. This will be done by feeding diets deficient in other elements (eg. Mg) by varying the type of carbohydrate fed, and by altering the protein content of the diet.

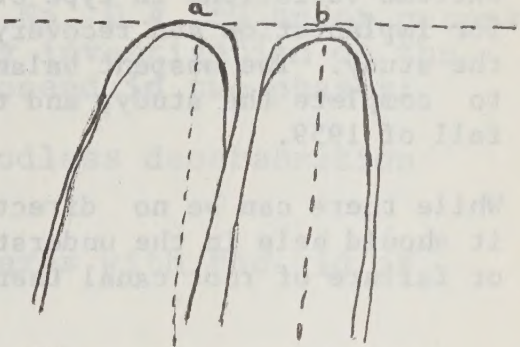
Also by following morphological characteristics of the teeth and related caries scores through several generations of animals, we hope to learn more of the genetic influence on these factors.

Appendix I

GRANTEE'S PROGRESS REPORT - D. A. 73 - to 31 January, 1959

Short Title: Study of inflammatory response to sterile glass cannulae in connective tissue.

Summary: In endodontics, root canals cannot always be obturated at the exact level of the "apical foramen". The shapes of the canal and the surface of the root apex preclude this possibility in most instances. Schematically, the canal would have to be unlarged to diameter a b, before the complete obturation could occur.



Less enlargement would result in obturation at a constriction distal to the anatomical root apex. With immature teeth a similar situation may exist - the walls of the canal being slightly or markedly divergent proximally in the apical half of the root (the so-called blunder-bus canal). A terminal bifurcation of the canal might be responsible for failure to obturate completely, and lateral canals are generally impossible to obturate completely. Aside from strictly anatomical problems, the necessity of relying on radiographs for observation makes consistently accurate results impossible. However, most root canal fillings appear to succeed clinically in spite of small discrepancies. Many writers have concluded on the basis of clinical experience and histological preparations that the cements dentinal junction is a good level at which to end the preparation and filling. This is often well within the canal. Obviously this leaves in many cases a narrow unfilled tract between the end of the filling and the end of the tooth. This is certainly true where part of a terminal bifurcation remains unfilled. With the blunder-bus canal the unfilled portion (assuming that root resection or a proximally placed filling are not carried out) is much wider and usually a little shorter. No clinical problem occurs with anterior teeth since they are easily resected, if necessary, to be the level of complete obturation. With many posterior teeth however and frequently with newly-erupted anteriors surgery is difficult or impossible.

With this as a background we determined to create a somewhat analogous situation in animals so that the influence of size and shape of the unfilled portion on inflammatory response might be studied.

It has been reported that whereas hollow tubes of glass implanted aseptically in the peritoneum of the rabbit (Rickert and Dixon) produced an extensive inflammatory response solid rods of the same material produced no foreign body reaction. If it can be shown that a solid, polished, inert rod implanted in connective tissue or intraperitoneally (guinea pig) produces a minimal response and that hollow tubes similarly prepared produced an inflammatory response of a much higher order, then probably a very short

tube with a very wide lumen would produce little or no response, the lumen either filling with granulation tissue or permitting sufficiently rapid interchange of accumulation fluid to prevent toxic material from forming in sufficient quantities to set up a response. Again it is possible that a tube with a very fine bore might produce no response, the available space for accumulation of toxic products being too small. It seems reasonable to believe that both the length and the diameter of the canal might influence the response. We prepared a number of tubes and rods for implantation which should permit us to establish whether or not this hypothesis is true. To date no specimens have been recovered for histological study. The initial operations, were made to determine the response to the more extreme variations in type of implant, to establish a suitable technique for implantation and recovery, and to determine if it was feasible to continue the study. The unspent balance of the present grant should be sufficient to complete the study, and the probable date for completion will be the fall of 1959.

While there can be no direct carry-over to clinical practice in this study, it should help in the understanding of one factor influencing the success or failure of root canal therapy.

Submitted 6th February, 1959

Robert L. Tegart, D.D.S.

NATIONAL RESEARCH COUNCIL

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

January, 1959

Subject: Basic oral reflexes

Grantee: R.D. Haryett

PROJECT: D.A. 69

Amount of Grant: \$2,300

Purpose:

Refer to report Grant No. D.A. 69 under purpose, paragraph C. To carry out an exploratory investigation on the basic oral reflexes. This study is to proceed in two phases:

Phase a: To develop a technic for bloodless decerebration of the experimental animal.

Phase b: To study the basic oral reflexes with the aid of electromyography.

Experimental Procedure:

It was decided to develop a technic of decerebration of anemia on cats first before applying this method on the monkeys. The procedure consists of two steps: 1st - ligation of the carotid arteries, second ligations of the basilar artery. (Fig. 1). This method is approximately the same as outlined in the studies of Pollock and Davis.*

Adult cats were anaesthetized with chloroform. Chloroform was used rather than ether because of the possibility of sparks from the dental hand piece and engine. As soon as the surgical stage was reached, the cat was placed on a specially designed operating table. The trachea was cannulated. For the remaining part of the operation chloroform was administered on a gauze sponge applied to the side tube of the cannula.

By blunt dissection, lateral and deep to the trachea, the right and left carotid arteries were exposed and ligatured tightly. The basilar artery was ligated in the following method. (Fig. 2).

* Pollock L.J. and Davis, L.E. Studies in Decerebration. 1. A method of decerebration. Archives of Neurology and Psychiatry 10: 391-398. 1923.

The animal's head was securely fixed and the jaws were widely separated by a special clamp. The tongue was held outside the mouth by means of a ligature through the tip of the tongue. A median line incision was made through the entire length of the soft palate commencing at the posterior edge of the hard palate. The free edges of the soft palate were then tied laterally by ligatures to give as much access as possible. The mucuous membrane and muscles were separated and retracted from the base of the skull as far posteriorly as the anterior border of the foramen magnum and laterally to expose the tympanic bullae. The prominent tympanic bullae serve as useful landmarks in localizing the point of entry through the bone. Opposite the middle of the tympanic bullae one reaches a level in the pons at about the exist of the fifth cranial nerve from the pons.

A large acrylic bur was used to trephine through the base of the skull (ventral surface of the body of the sphenoid, basiasphenoid, and basioccipital bones). This method worked well since it tended to stop any bleeding from the edges of the bone. The cutting was continued until a very thin layer of bone remained which could easily be removed with the beaks of pliers. Beneath the membrane-like layer of bone was located the dura mater. The basilar artery was visible lying under the dura mater. A sharp instrument was used to make a small opening and allow the cerebrospinal fluid to escape. The objective is to ligature the basilar artery opposite the middle of the tympanic bullae which is the level of the exit of the fifth cranial nerve from the pons.

Shortly after both carotid and basilar arteries are ligated, the cat assumes the characteristic posture of decerebrate rigidity.

Results and Conclusion:

The method described above has been performed on six cats with reasonably good success. However, the experimenter wishes to improve his technic before decerebrating the monkeys, which are not too plentiful. It is felt that this method can be used and will be very helpful to continue phase B i.e. to study basic oral reflexes.

The writer wishes to express his sincere thanks to Dean H.R. MacLean and Dean S. Hamilton of the Faculty of Dentistry, Dr. J. Pearce, Head of the Department of Physiology and Pharmacology and my assistant Mr. Percy Kirby, Dentistry Three, for their cooperation and generous assistance.

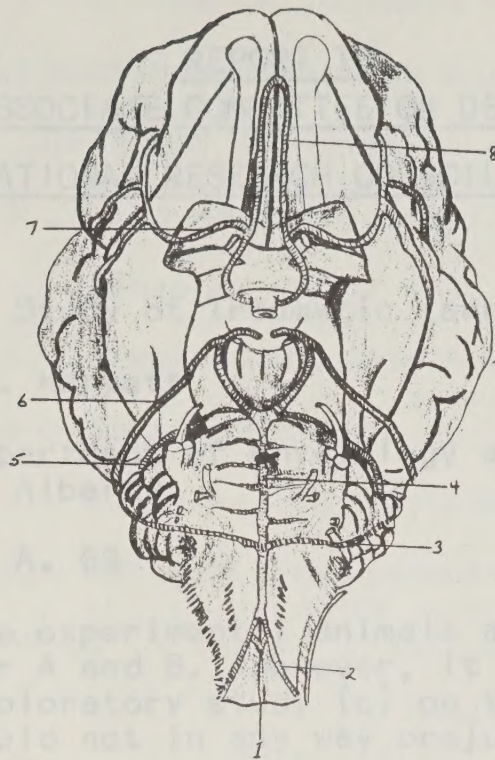


Figure 1

Drawing of the basal surface of the cat's brain, showing the arrangement of the vessels constituting the circle of Willis. 1. Anterior spinal artery; 2. vertebral artery; 3. posterior inferior cerebellar artery; 4. basilar artery; 5. posterior superior cerebellar artery; 6. posterior cerebral artery; 7. middle cerebral artery; 8. anterior cerebral artery.

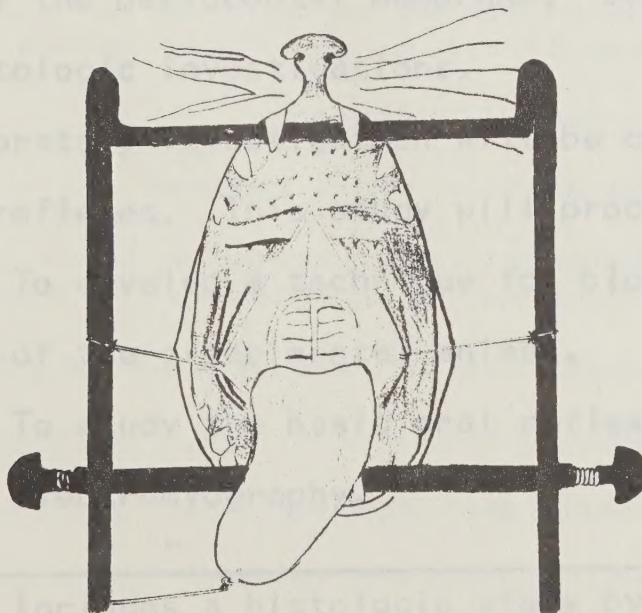


Figure 2

Sketch of the jaws of the cat fixed in the clamp. The mucous membrane is retracted to expose the tympanic bullae. The trephine opening in the bone is located between the bullae. The basilar artery is visible on the ventral surface of the pons.

REPORT TO
THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
NATIONAL RESEARCH COUNCIL OF CANADA

Subject: The Study of Traumatic (surgical) Tooth Movement.

Author: R.D. Haryett
*

Laboratory: Department of Physiology and Pharmacology, University of Alberta.

Grant No. D. A. 69

Purpose: The experimental animals are to be used specifically for A and B. However, it is hoped to do a further exploratory study (c) on the same animals, which would not in any way prejudice the findings in A or B.

A. The prime objective and first phase is to study the tendencies of relapse in traumatically rotated incisors of rhesus monkeys. This phase is being carried out by R. D. Haryett.

B. The second phase will include the study of the effects of traumatic tooth rotations on the pulp vitality and ultimate integrity of the periodontal membrane. Dr. McMurchy will carry out the histologic investigations.

c. An exploratory investigation will be carried out on the basic oral reflexes. This study will proceed in two phases:

Phase (a): To develop a technique for bloodless decerebration of the experimental animal.

Phase (b): To study the basic oral reflexes with the aid of electromyography.

* The grant includes a histologic study by Dr. K. A. McMurchy; the study to be carried out by Dr. McMurchy is to be completed after the active phase when the animals have been sacrificed.

EXPERIMENTAL PROCEDURE: (see report of January 1958 on Grant No. D. A. 69)

Adult rhesus monkeys were anaesthetized by intraperitoneal injections of Nembutal. A level of 25-30 mg. of Nembutal per kilogram of body weight gave adequate relaxation for an operating period of three hours. One three hundredth grain of atropine was added to and injected with the Nembutal to reduce salivation. The monkeys were placed in a specially designed small dental chair for operative procedures.

The operative procedures were carried out on each experimental animal in the following steps: (Steps 1, 2, and 3 were illustrated in report of January 1958)

- Step One:
- a. Maxillary and mandibular impressions for study models
 - b. Roentgenograms of maxillary incisors
 - c. Brass wire separation between the teeth to be banded
 - d. Photographs

- Step Two:
- a. Separation wires removed
 - b. Bands fitted on the maxillary incisors, canines, one bicuspid, and first molars
 - c. separation replaced

- Step Three:
- A. Separation wires removed
 - b. Cementation of bands on teeth except the incisors to be rotated.
 - c/ Insertion of passive .020 round arch
 - d. Photographs

- Step Four.
- a. Round arch removed
 - b. Maxillary left lateral incisor rotated mesio-labially
 - c. Maxillary right central incisor rotated mesio-labially.
 - d. Bands cemented on rotated incisors

- e. .020 round stainless steel passive arch ligated with .012 ligature wire (Fig. 1 and 2.)
- f. Photographs and roentgenograms of the maxillary incisors

Step Five:

- a. Monkeys are checked every 6-8 weeks for loose bands and ligatures. Progress roentgenograms of the maxillary incisors were taken. (Fig. 6)
- b. Six months after the teeth were rotated, all bands were removed. Roentgenograms, photographs, (Fig. 3) and impressions for study models were taken.

Step Six:

- to be carried out in approximately 3 months.
- a. Final impressions will be taken prior to sacrificing the monkeys.
- b. Just prior to sacrificing the monkeys, a bloodless decerebration operation will be performed to evaluate its effectiveness for future studies on the basic oral reflexes.

Step Seven:

- a. to be carried out by Dr. K. A. McMurchy after the monkeys are sacrificed. Step seven will involve the histologic aspect of the study.

RESULTS AND CONCLUSIONS:

The final roentgenograms and study models have not been obtained to complete the data. Consequently, there has not been any attempt as yet to analyse the data and make conclusions. However, one is able to make a few general observations.

1. It is very difficult to rotate an incisor tooth and at the same time maintain its vertical relation in the dental socket. If the root form was a perfect cone fitting in a cone-type of dental socket, the rotation would be simple. However, since the root form is not an ideal cone and is usually slightly curved, there seems to be a tendency when rotating an incisor for the tooth to emerge from its socket. (Fig. 4.) The same effect would be experienced if one was twisting a corkscrew backwards from a piece of wood and at the same time attempting to prevent the corkscrew from emerging from its hole.

In two attempts at rotation, an incisor was inadvertently extracted as the crown popped through the beaks of the forceps.

Further incidences of this nature were prevented by fitting a small piece of a rubber cork within the confines of the beaks.

In another over-zealous attempt to prevent extrusion of an incisor during rotation, the crown was fractured from the root. (Fig. 5.)

2. As the tooth is rotated, the axial inclination of the tooth changes due to the irregular root form. It seems apparent that when an attempt is made to align a rotated tooth in the dental arch by rotation alone, the alteration in axial inclination would create a lingual, labial, mesial, or distal angulation. (Fig. 4.)

3. No attempt will be made to assess the findings in the roentgenograms and on the study models until the complete records have been obtained.

4. It is believed that a reasonably good technique has been developed for carrying out oral experimental procedures on the monkey.

The writer wishes to express his sincere thanks to Dean H. R. MacLean and Dean S. Hamilton of the Faculty of Dentistry, Dr. J. Pearce, Head of the Department of Physiology and Pharmacology, and my assistant Mr. Percy Kirby, Dentistry Three, for their co-operation and generous assistance.

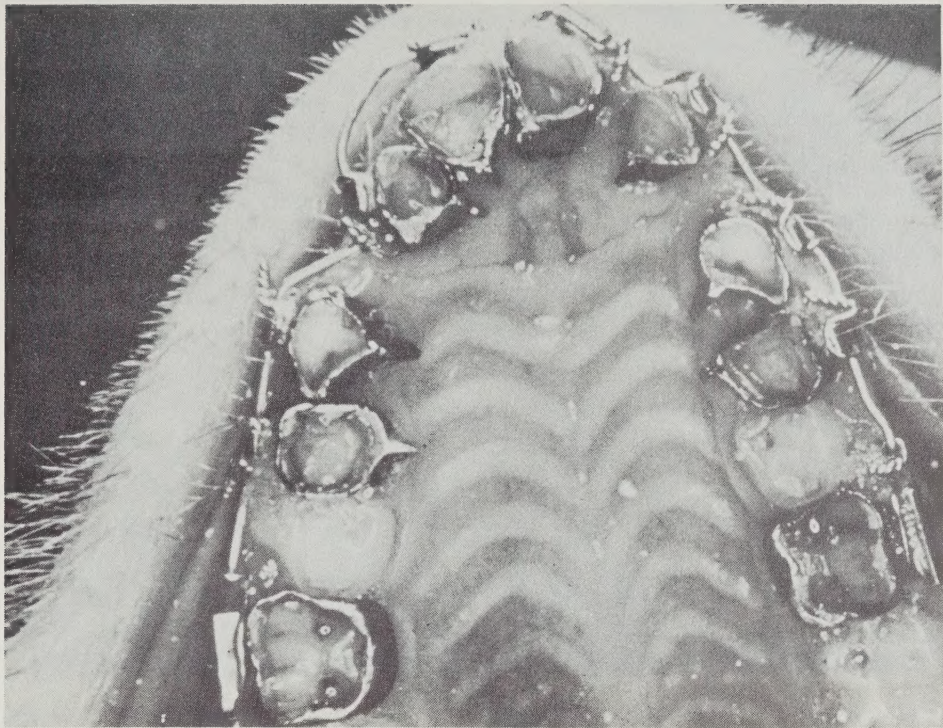


FIGURE 1. Occlusal view of .020 round stainless steel passive arch ligated after tooth rotation.



FIGURE 2. Anterior view of .020 round stainless steel passive arch ligated after tooth rotation.

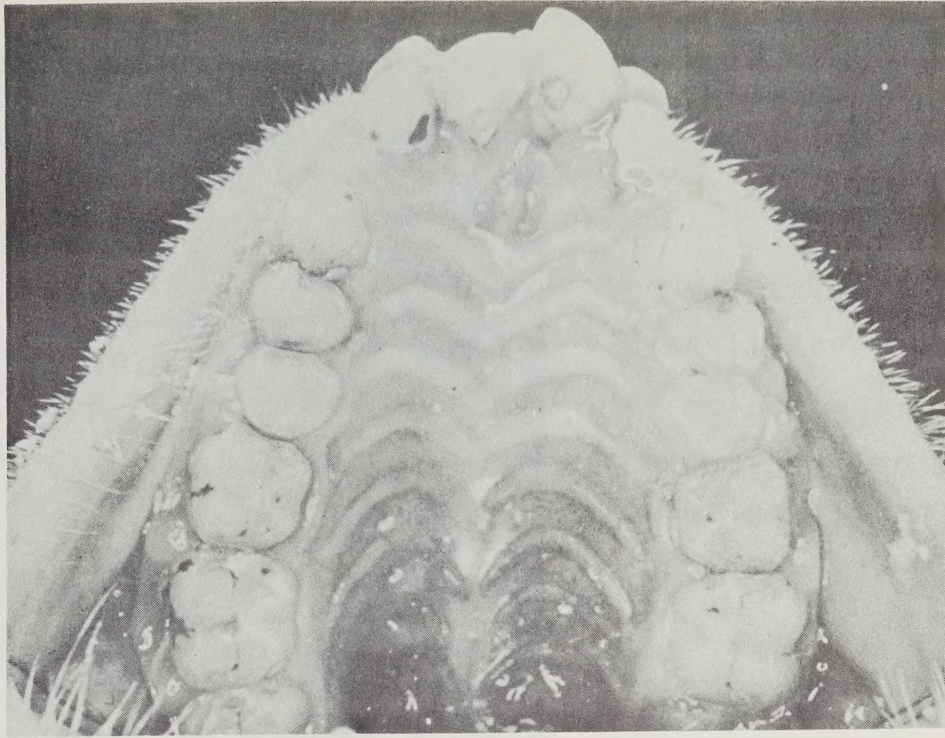


FIGURE 3. Occlusal view of maxillary dental arch after band removal. Note tooth malalignment. Resulting from rotation.

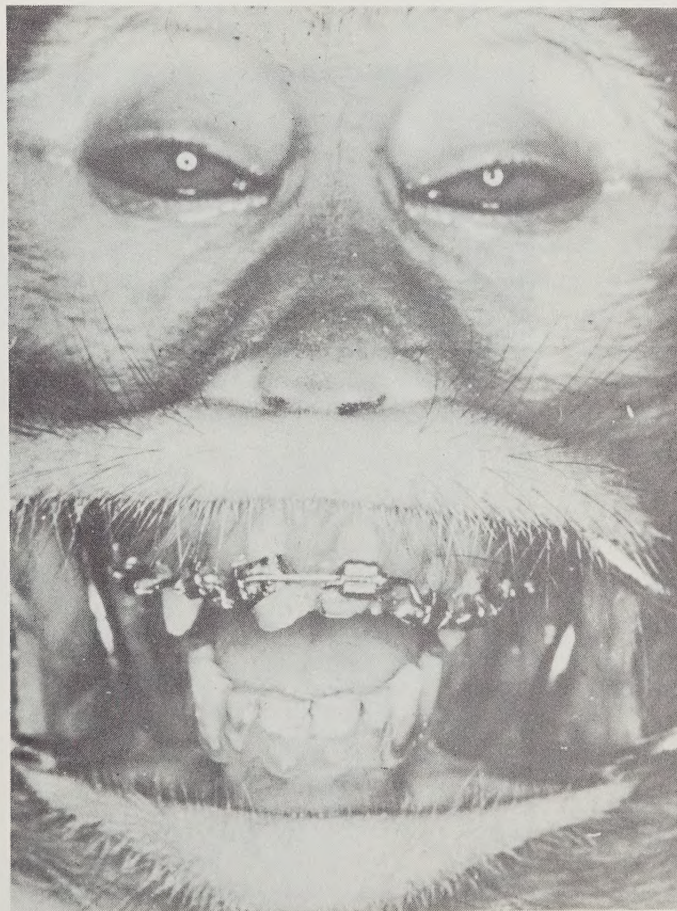


FIGURE 4.

Note the extrusion and altered axial inclination of right central incisor which occurred during the rotation.

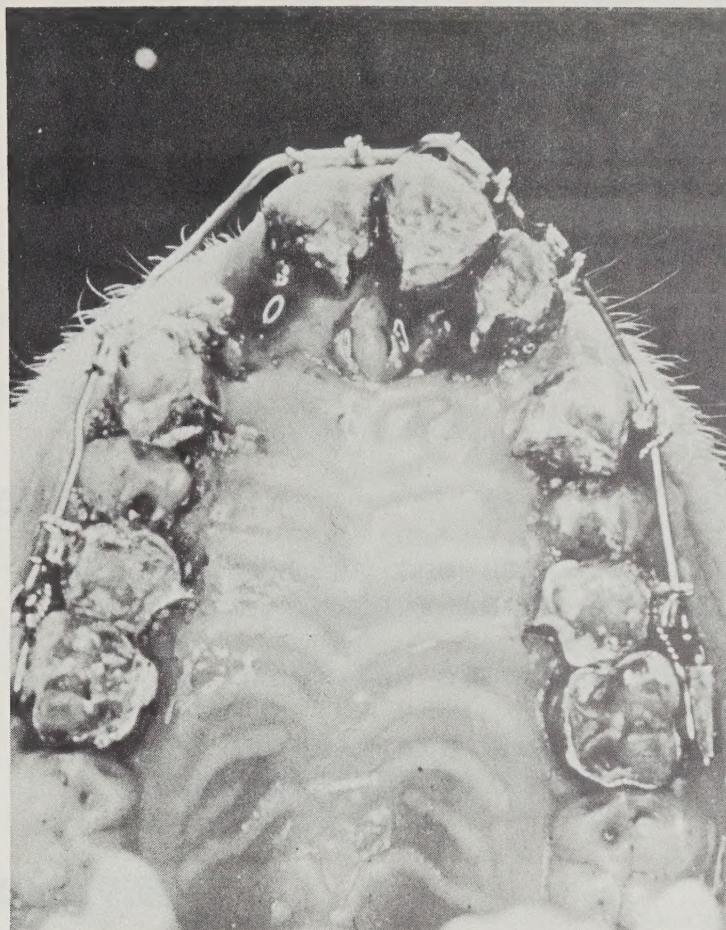


FIGURE 5

Occlusal view. Crown of maxillary right lateral incisor was fractured from root at gingival margin during attempt of traumatic rotation.

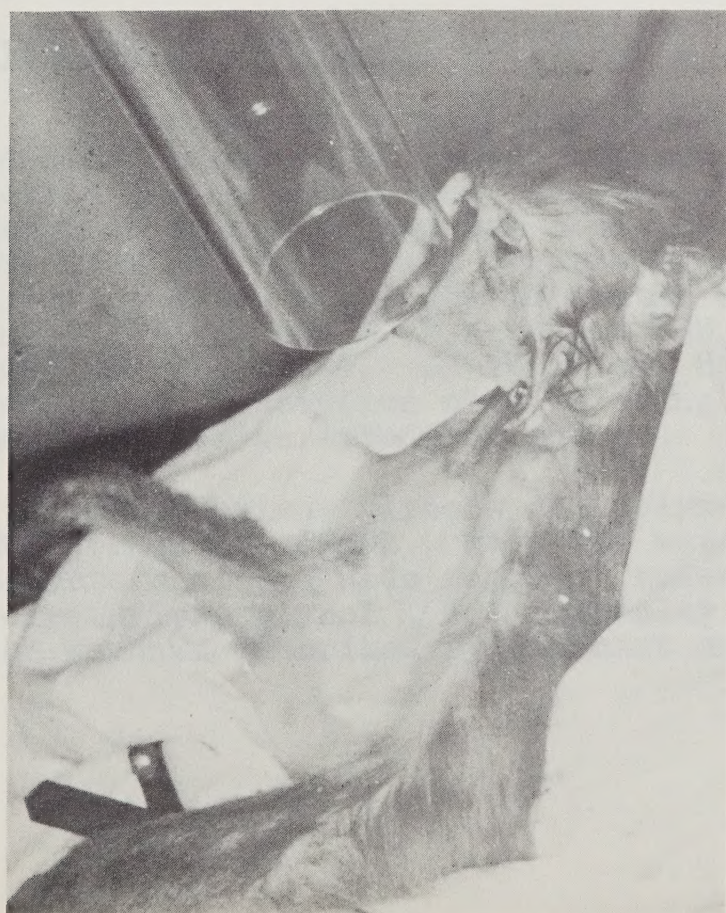


FIGURE 6

Taking of oral Roentgenograms.

Appendix K

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January, 1959

SUBJECT: A study of resorption of enamel dentin and cementum.

GRANTEE: Dr. K.A. McMurchy

PROJECT: D.A. 67

AMOUNT OF GRANT: \$3,000

OBJECT:

1. To examine the surface of resorbed dentin in extracted human primary teeth for evidence of border striations.
2. To study the osteoclasts in the area.
3. To investigate those aspects of dentin composition and maintenance which may be revealed by a study of its resorption.

PROCEDURE:

Human primary teeth were used. Many of these teeth were diseased and were extracted because of various forms of pulpitis. Roentgenographically many showed signs of undergoing resorption. A case history was kept for each tooth.

HISTOLOGICAL TECHNIC:

In an effort to minimize distortion of the specimens due to differences in shrinkage between the soft tissues and the dentin during processing, polyethylene glycols were used for dehydrating and embedding. This decision was based on work done by Miles and Linder¹ who reported that blocks of liver shrank 11.7% in paraffin, 10.8% in double embedded paraffin wax, 6% in celloidin, and 4.7% in Nonex 63B, etc. (polyethylene glycol 1000 monostearate). In addition, Hancock² has reported superior results in resorption studies using polyethylene glycol 1000 monostearate as embedding media.

Polyethylene glycols are polymers with the general formula $\text{HOCH}_2(\text{CH}_2\text{OCH}_2)_x\text{CH}_2\text{OH}$. They are water soluble, hygroscopic materials with a wide range of molecular weights. In this study polyethylene glycol 1540* molecular weight was used following the technic described by Rinehart and Abul-Haj.³

This was supplanted by the use of polyethylene glycol 1000 and polyethylene glycol 1000 monostearate** as used by Miles and Linder. Difficulty was experienced keeping the polyethylene glycol 1000 molten at 39°C, so it was replaced by polyethylene glycol 600*, which because of its lower molecular weight, should have better penetrating ability and should be a better dehydrating agent because of increased hygroscopicity†. Staining results proved to be superior with the material embedded in polyethylene glycol 1540, so it is now routinely used. Details of the histological technic are as follows:

1. Fixation - Buffered neutral 10% formalin solution.
2. Wash in tap water, rinse in distilled.
3. Decalcification - formic acid-sodium citrate.
4. Wash in tap water, rinse in distilled.
5. 50% aqueous solution of polyethylene glycol 600 for 30 minutes, at 49°C.
6. Pure polyethylene glycol 600, two changes, 30 minutes at 49°C.
7. Equal parts polyethylene glycol 600 and 1540 for 30 minutes at 49°C.
8. One part pg 600, three parts pg 1540 for 30 minutes at 49°C.
9. Pure pg 1540, overnight at 49°C.
10. Embed in fresh pg 1540 and allow to harden at room temperature or in the refrigerator.
11. Trim blocks and mount as with paraffin, avoiding moisture. Cut at once for best results. The knife tilt should be greater than that used for paraffin. Sections may be cut at 4 - 6u. Ribbons form readily and are cut into individual sections.
12. Float individual sections out on tap water, and pick up on clean slides. No affixative seems to be necessary. Dry overnight in oven at 40°C.
13. Sections were stained with Alum Haematoxylin Gosin, Heidenhain Haematoxylin-Van Gieson, Mallory Aniline Blue, MacManus P.A.S., and Foot-Hortega sliver stain.

RESULTS:

The material proved to be a very good source of areas of active resorption of dentin. Eighteen teeth have been sectioned thus far, nine of which have yielded resorption areas worthy of study. The soft tissue adhering to the outer surface of the extracted tooth was disrupted in most instances, so cell detail was observed only in areas where resorption was occurring on the pulp wall. The soft tissue in this area was relatively undisturbed by the physical tensions of extraction.

Resorption of enamel, cementum, mantle dentin, and pre-dentin have not been studied thus far. Since the resorption areas studied involved the pulp wall, the type of dentin was circumpulpal, secondary, or reparative.

The polyethylene glycol 600 - 1540 dehydrating and embedding method has been very satisfactory and we are now using it routinely in our laboratory for the sectioning of both decalcified material and soft tissue. The method is also being used in the Anatomy department for studies on fat in muscle. It would also be suitable for studying myelin sheaths of nerves since the myelin is preserved and stains well in ordinary H & E preparations.

OBSERVATION:

Decalcified specimens cut at 4-6 μ and stained with five stains were observed with the bright field and phase contrast microscope.

(1) The surface of resorbing dentin. The surface of resorbing dentin varies considerably in appearance depending on the cells overlying it. Where osteoclasts are present, typical Howships lacunae are formed. These are occupied by the osteoclast cytoplasm. A "striated border" is seen at the junction of the cytoplasm and the dentin in some cases. With silver stains and fibrils of the dentin matrix can be seen to end rather abruptly, short of the striated border region. These fibrils are much thicker than the elements of the striated border and are evident particularly on surfaces which are parallel to the direction of the dentinal tubules. They also appear in areas where vacuoles touch the resorbing dentin surface.

(2) The osteoclasts. These multinucleated cells were observed to have all the morphological characteristics ascribed to true osteoclasts⁵. Their appearance varied considerably, probably due to different states of functional activity. While an arrangement of these cells in age or functional order must

be purely subjective it is felt that the earliest signs of resorption are associated with masses of fibroblast-like nuclei which appear close to the dentin surface. Howships lacunae in the best preparations were seen to be filled by obviously multi-nucleated cells with a very finely granular cytoplasm which in H & E and Heidenhain-Van Gieson stained slides appeared to blend almost imperceptibly into dentin. No distinct "striated border" could be seen in most of these cells nor were extracellular vacuoles prominent in these areas. The third groups of cells displayed "striated borders" intracellular vacuoles, granules, and nuclei with thicker nuclear membranes and larger nucleoli. Associated with these were extracellular vacuoles or spaces.

In silver stained specimens many fibers appear to protrude from the resorbing surface. In some areas bits of the thick fibers seemed to be present in or go through the osteoclast cytoplasm. In general, however, the dentin surface is devoid of protruding fibers only in areas where the osteoclast cytoplasm is in contact with the dentin. Thick fibers, presumably collagen, often surround the osteoclast cytoplasm on the pulpal side (Fig. 6).

Observations on section stained with PAS yielded inconclusive results. The only areas to stain rosy red were areas of carious dentin and a few inflammatory cells in the pulp area. The dentin and the osteoclasts stained pale pink. The only significant fact was that the osteoclast cytoplasm was deeper pink close to the dentin.

The Aniline Blue, stained slides did not show the relationship of the collagen fibers to the resorbing dentin surface as well as the Van Gieson and silver stained preparations.

DISCUSSIONS AND CONCLUSIONS:

The multinucleated cells involved in dentin resorption are morphologically indistinguishable from the osteoclasts associated with bone resorption (Fig. 1).

Striated borders occur at the junction of some of these cells with the surface of the dentin (Figs. 2 & 3). The direction of the striations is radial and bears no relationship to the direction of the dentinal tubules in the area (Fig. 2). The striations appear to be part of the osteoclasts, possibly microvilli involved with secretion activity, possibly physical phenomena associated with withdrawal of the cell from the dentin surface after functional activity.

This and their morphology (Fig. 4), suggests motility of the osteoclasts which may also explain the "pushed aside" appearance of the large collagen fibers around these cells (Fig. 5). The large extracellular vacuoles may be tissue fluid accumulations brought about by the withdrawal of osteoclast cytoplasm from the area. Fibroblasts may then move into these areas and affect the attachment of connective tissue to the resorbing surface. This stage would be very difficult to distinguish histologically from the earliest stage of resorption.

It is by no means clear that some of the large fibers are not Korff's fibers which were once embedded in the dentin. If this is the case it would support the evidence of recent electron microscope work that the Korff fibers "pass directly through the layers of circumpulpal predentin as compact bundles" and, "there is no evidence of any close anatomical association between Korff's fibers and the network of fine fibrils extending over the entire predentin area" which, it is suggested may "arise from an afibrillar substance in the subodontoblastic region". It is a possibility that the osteoclast may be capable of destroying the fine fibrils as well as the ground substance but is unable to cope with the large Korff fibers which would be left protruding from the dentin surface.

It is proposed to continue this study but no further assistance is being requested. A final report will be submitted.

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Figure 1

Resorbing Dentin and Osteoclast. Stained with Heidenhain-VanKieson stain, viewed with bright field x 970

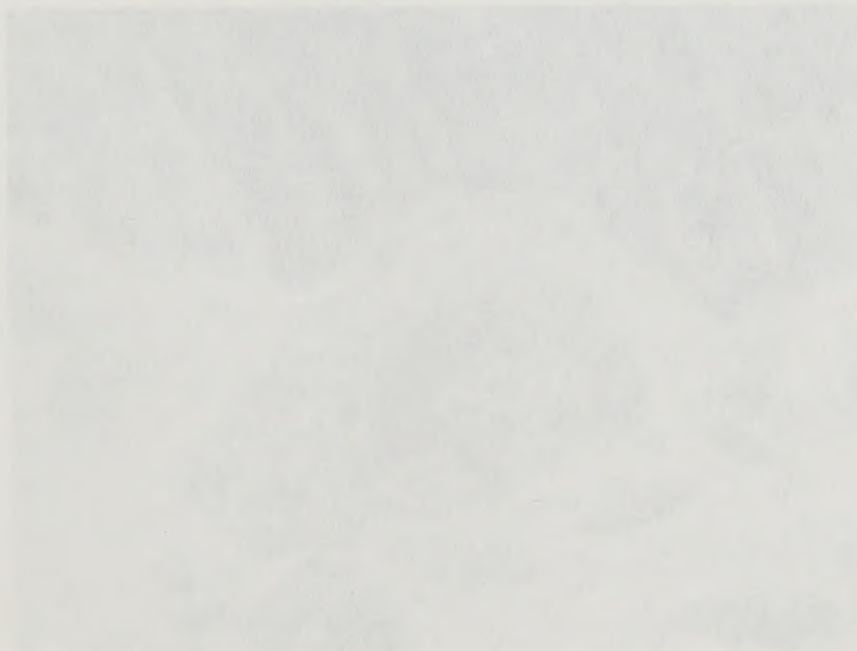


Figure 2

Same cell as Fig. 1. Viewed with Phase contrast x 970 showing striated border

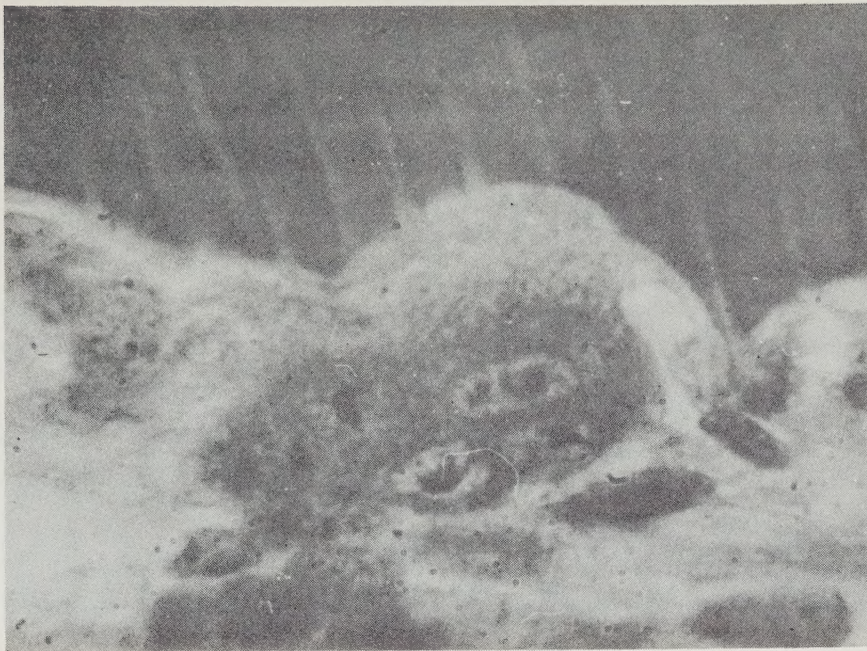


Figure 1

Resorbing Dentin and Osteoclast. Stained with Heidenhain-VanGieson stain, viewed with bright field x 970

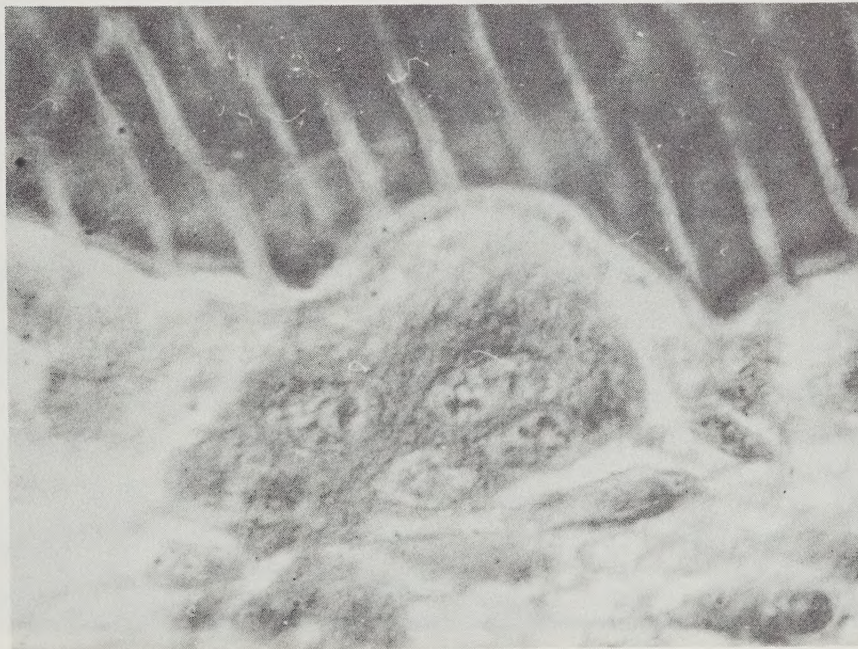


Figure 2

Same cell as Fig. 1. Viewed with Phase contrast x 970 showing striated border

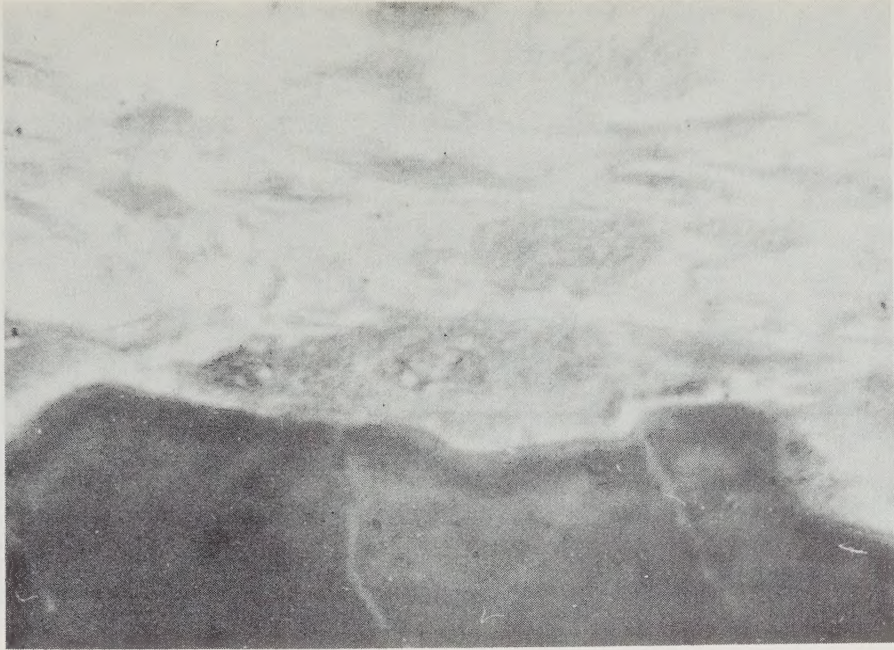


Figure 3

Osteoclast on reparative dentin surface. Heidenhain-Van Gieson stain. Phase contrast x 900

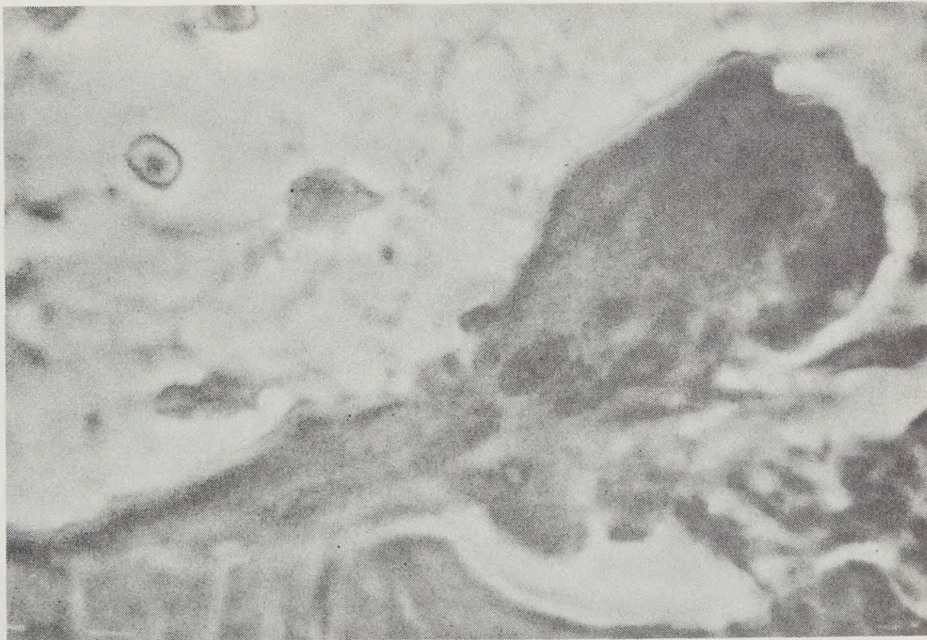


Figure 4

Osteoclast with pulpal projection Heidenhain-Van Gieson
Phase contrast x 900

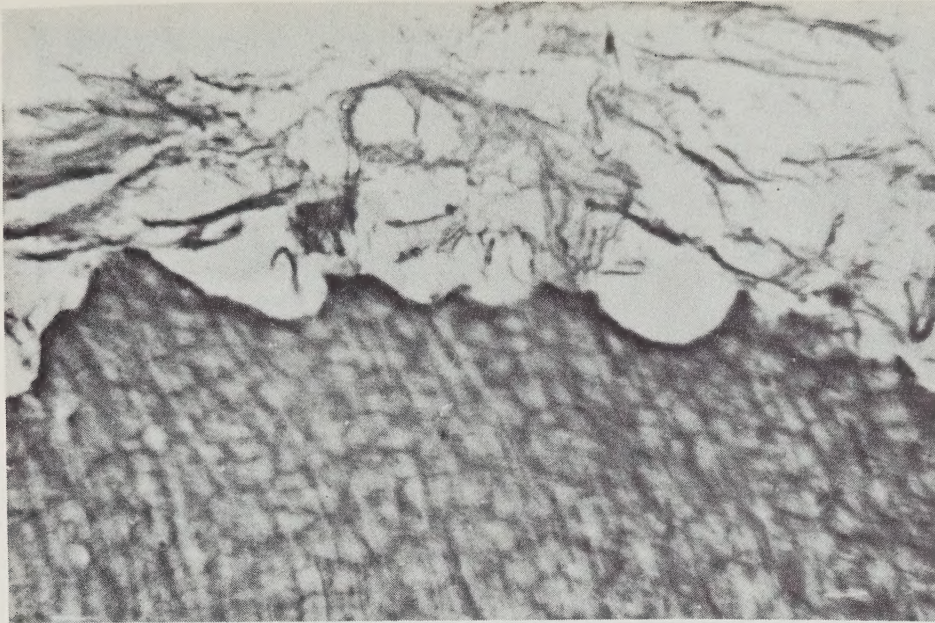


Figure 5

Resorbing surface Foot Hortega silver stain. Large clear spaces on Dentin surface are Osteoclast cells.

Bright field x 400

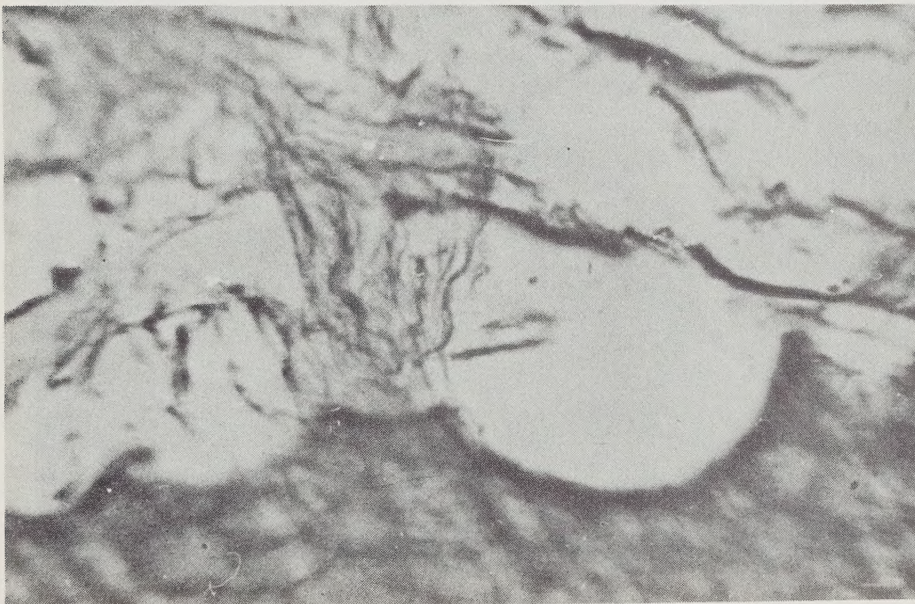


Figure 6

Same as Fig. 5 x 900. Osteoclast cytoplasm faintly visible. A large vacuole is present to the right. Resorbing surface is quite uniform.

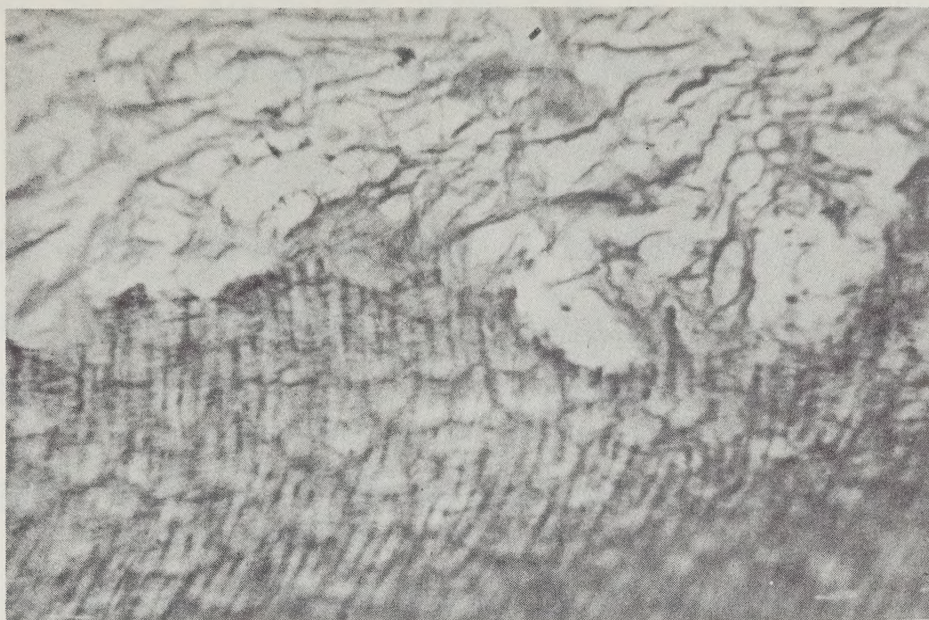


Fig. 7. Silver Stain showing fibers in Dentin and Protruding from a resorbed surface. Bright Field X400.

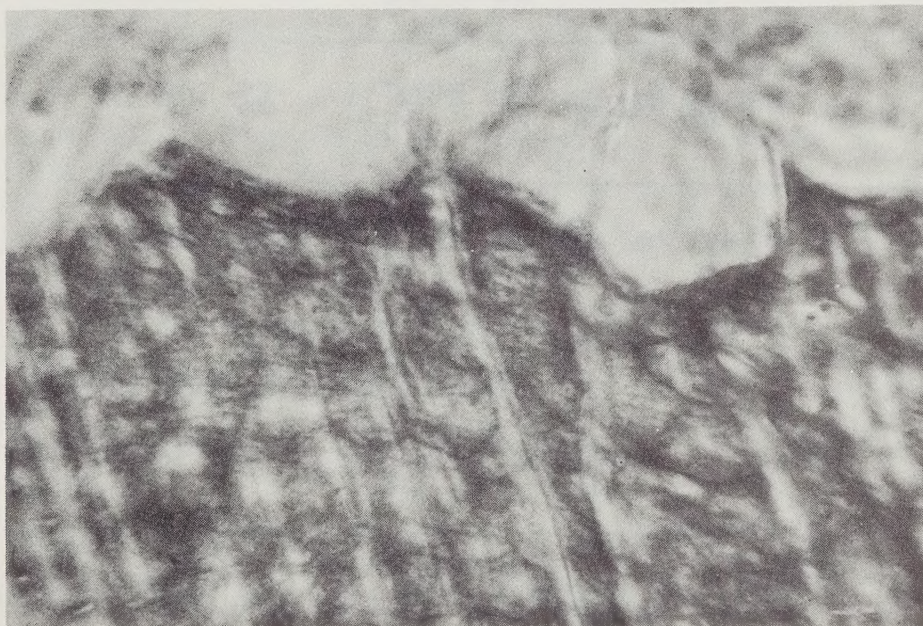


Fig. 8. Silver Stain. Phase contrast showing fibrils in Dentin and what may be fibers parallel to the Tubules. X900

Appendix L

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH NATIONAL RESEARCH COUNCIL

Subject: The effect of BAPN on newborn rats
Grantee: L.F. Belanger
Project: D.T. 52 Amount of Grant: \$4,500.00

TREATMENT: 200 mgm in 10 cc H₂O (2% solut. BAPN fumarate)
Dose: 0.2 ml. s.c.p.d.

Gr. I 4/4 at 2 days: 0.1 cc. p.d. 4 days

Gr. II 4/4 at 3 days: 0.15 cc. p.d. 4 days

Gr. III 4/4 at 5 days: 0.15 cc. p.d. 8 days

RESULTS: (a) Length of trunk (vertebral column)
at autopsy:

Gr. I : BAPN 60 mm. average
C. 66 " "

Gr. II : BAPN 60 mm. average
C. 70 " "

Gr. III : BAPN 72 mm. average
C. 77 " "

(b) Weight at autopsy:

Gr. I : BAPN 7.6 gm.
C. 11.5 gm.

Gr. II : BAPN 6.7 gm.
C. 13 gm.

Gr. III : BAPN 13 gm.
C. 15 gm.

(c) Autopsy remarks:

Gr. I : 2 animals died between injection of tracer Ca^{45} and autopsy. Were radioactive all over. Rigid and stretched out.

All animals of Grs. I and II were considerably redder than controls. There were hemorrhages over limbs in vicinity of joints particularly, and also in the face.

The skin was dry, wrinkled, "parcheminée" like in mongolism.

Gr. III showed much less change in appearance.

(d) Stained sections: CARTILAGE: (AP-PO) General depletion in upper extremity of cartilage as compared to lower. Irregular cell disposition in cartilage.

BONE: Many changes apparent: thinning, deformation, exostoses and cartilage metaplasia in diaphysis, mid-diaphyseal fractures. Sub-periosteal hemorrhages; periosteal hypertrophy.

SOFT TISSUES: The heart, kidney, lung and aorta showed nothing remarkable in H.P.O. stained sections. The thymus is possibly decreased in size with disappearance of medulla. Skin aedematous; hemorrhages.

TEETH: Irregular, wavy, deformed teeth. Decrease of stainable dentine at dentino-enamel junction. In polarized light and phase contrast, this area is dark. The enamel shows hyperplasia of ameloblasts in spots and production of enamel pearls. In other places, the ameloblasts appear clear, degenerating.

(e) Phase contrast (unstained mineralized celloidin sections): In bones, principally in alveolar membrane bone, precipitate of round masses in spaces between trabeculae. Also present in teeth, between odontoblasts and pre-dentine or in pulp cavity.

(f) Polarized light: the precipitate is birefringent.

(g) Ca^{45} autoradiography: the precipitate is Ca^{45} positive.

CONCLUSIONS: (1) Lathyrism effects growth, decreasing length and body weight of the animal.

- (2) The effect is more pronounced when treatment is initiated at 2 and 3 days than at 5 days in rats.
- (3) The skeletal tissues of mesodermic origin, cartilage, bone and dentine are particularly affected.
- (4) The lesions consist of loss of chondroitin sulfate from the upper extremities particularly, softening of articular surfaces, thinning of bones, fracture of mid-diaphysis. The periosteal hypertrophy, cartilage metaplasia and exostoses seem to be compensatory.
- (5) The precipitation of round crystalline masses containing Ca^{45} in the vicinity of mineralizing surfaces has been confirmed. They seem to indicate a defect in utilization of Ca ions through some defect of bone forming cells (osteoblasts). The thinning of bone seems also related to a defect of the function of the osteocyte resulting in decrease in mineralization.
- (6) The enamel organ and skin are also affected.
- (7) No lesions were found in the heart, large blood vessels and kidney under present experimental conditions.
- (8) The bone and cartilage syndrome obtained here with young rats and BAPN reproduces that previously obtained in chicks fed lathyrus seeds.

APPENDIX M

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

SUMMARY OF APPLICATIONS FOR SUMMER DENTAL RESEARCH ASSISTANTSHIPS

<u>Name</u>	<u>Age</u>	<u>School</u>	<u>Year</u>	<u>Remarks</u>	<u>Recommended by</u>
Andreas, F.P.	32	Toronto	3rd	Dental Research Assistant under Dr. K.J. Paynter (1958). Ranked first in class in first two years.	K.J. Paynter G. Nikiforuk A.M. Hunt
Boily, J-G.		Montreal	3rd		G. Pelletier Z. Mezl J-P. Lussier
Brean, G.J.	26	McGill	3rd	B.Sc., Geology, St. F.X., 1953. Instructor, Chemistry Laboratory, St. Francis Xavier Univ.; Supervisor, chemical company; research on corrosion, 1953-56.	
Buckley, K.G.	26	Dalhousie	2nd	B.Sc., St. F.X., 1954. B.A., St. F.X., 1955. Ranked 2/13 in first year.	A.K. Reynolds J.D. McLean R.L. de C.H. Saunders
Fletcher, R.	25	McGill	2nd	Dental Research Assistant under Dr. Leblond (1958).	
Germain, P.	22	Montreal	1st		
Goring, N.L.	29	McGill	1st	B.Sc., Agriculture, McGill, 1957. Laboratory work (summers 1955-58).	H.G. Dion R.H. Ozburn

<u>Name</u>	<u>Age</u>	<u>School</u>	<u>Year</u>	<u>Remarks</u>	<u>Recommended by</u>
Hechter, T.	29	Alberta	3rd	B.Sc., Manitoba, 1956. Instructor in organic chemistry, one year. Ranked 4/30 and 9/26 in first two years.	K.A. McMurchy
Jow, R.B.	24	Toronto	2nd	B.Sc., Physiology, U.B.C., 1957. 1 summer, research on strontium metabolism in the white rat. Ranked 1/78 in first year.	K.J. Paynter *A.M. Hunt J.E. Anderson
Kilvert, A.R.	22	Alberta	2nd	1956-57 (summers), Imperial Oil Co., routine tests. Ranked 3/28 in first year.	D.E. Florence R.C. McClelland K.A. McMurchy
Kirby, P.J.	29	Alberta	3rd	Dental Research Assistant under Dr. R.D. Haryett (1958). Ranked 16/26 in second year.	*C.R. Castaldi *R.D. Haryett K.A. McMurchy
LeBlanc, C.	24	Montreal	2nd	B.Sc., St. Joseph's Univ. Meteorological technician, 2 summers.	S. Sonea J-P. Lussier G. Gauthier
Levine, M.	22	Dalhousie	3rd	Demonstrator, Biology Laboratory, 2 years. 1958 (summer), Can. Dept. of Fisheries, field assistant.	*A.E. Hoffman D. Pelluet J.D. McLean

* Who is willing to supervise applicant if successful.

<u>Name</u>	<u>Age</u>	<u>School</u>	<u>Year</u>	<u>Remarks</u>	<u>Recommended by</u>
Marcil, A.	23	Montreal	2nd	Ranked 1/38 in first year.	J. Bussieres G. Gauthier J.P. Lussier
Meyer, W.C.	27	Alberta	2nd	1 summer, Physics Dept., Univ. of Alberta. Ranked 18/28 in first year.	D.H. MacDougall C.G. Duke K.A. McMurchy
Ngui-Kon-Sur, L.	21	McGill	1st		Y. Clermont H. Garcia-Arocha M. Banfill
Paszti, M.	32	Toronto	3rd	Dental Research Assistant under Dr. E.M. Madlener (1958).	*E.M. Madlener G. Nikiforuk K.J. Paynter
Robinson, B.W.	22	Toronto	2nd		J.D. Purves R.J. Godfrey J.E. Anderson
Trester, P.H.	19	Manitoba	1st		I.M. Thompson *I. Kleinberg H.W. Hart
Wallington, G.A.	21	Alberta	3rd	Ranked 10/26 in second year.	C.R. Castaldi K.A. McMurchy

* Who is willing to supervise applicant if successful.

<u>Name</u>	<u>Age</u>	<u>School</u>	<u>Year</u>	<u>Remarks</u>	<u>Recommended by</u>
Wasylchuk, M.	22	Alberta	3rd	Ranked 1st in class in first year and achieved first class standing in second year.	B.M. MacKenzie N. Basaraba
Waterbury, D.D.		Alberta	3rd	Summer, Forestry Div., Dept. of Agriculture. Ranked 6/26 in second year.	C.R. Castaldi
Weinlander, G.H.G.	28	McGill	2nd	Master's degree in Bus. Admin., Vienna. Lab. demonstrator, Chem., Sir George Williams College. Ranked first in class in first year.	M. Hebert J. McCutcheon H. Garcia-Arocha.

Appendix N

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointment (cont'd)

	<u>Term to Expire</u>
* 1. Dr. D.H. Copp, <u>Chairman</u> , Department of Physiology, University of British Columbia, Vancouver, B.C.	31 March, 1959
2. Dr. E.W.R. Steacie, <u>ex officio</u> , President, National Research Council, Ottawa 2, Ont.	- - -
3. Dr. R.F. Farquharson, <u>ex officio</u> , Vice-President (Medical), National Research Council, Ottawa 2, Ont.	- - -
4. Dr. D.L. Thomson, <u>ex officio</u> , Vice-Principal, McGill University, Montreal, P.Q.	- - -

Representing the Dental Profession

5. Dr. C. Castaldi, Faculty of Dentistry, University of Alberta, Edmonton, Alta.	31 March, 1960
6. Dr. C.D. Husband, Suite 110, Metro Block, Red Deer, Alta.	31 March, 1959
* 7. Dr. Jean-Paul Lussier, Faculty of Dentistry, University of Montreal, Montreal, P.Q.	31 March, 1960
8. Dr. R.J. O'Neil, 2525 Pine Street, Vancouver, B.C.	31 March, 1959

* Member of Executive

Term to expire

Representing the Dental Profession (cont'd)

- | | | |
|------|--|----------------|
| * 9. | Dr. A.G. Racey,
Faculty of Dentistry,
McGill University,
Montreal, P.Q. | 31 March, 1959 |
| 10. | Dr. C.H.M. Williams,
Associate Professor of Periodontology,
Faculty of Dentistry,
University of Toronto,
Toronto 5, Ont. | 31 March, 1960 |

Representing the Royal Canadian Dental Corps,
Department of National Defence

- | | | |
|-----|---|-------|
| 11. | Brig. K.M. Baird, <u>ex officio</u> ,
Director General of Dental Service,
Royal Canadian Dental Corps,
Department of National Defence,
Ottawa, Ont. | - - - |
|-----|---|-------|

Representing the Division of Dental Health,
Department of National Health and Welfare

- | | | |
|-----|---|-------|
| 12. | Dr. H.K. Brown, <u>ex officio</u> ,
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ont. | - - - |
|-----|---|-------|

Representing Dental Research,
Department of Veterans Affairs.

- | | | |
|-----|--|-------|
| 13. | Dr. L.A. Kilburn, <u>ex officio</u> ,
Director of Dental Research,
Department of Veterans Affairs,
Ottawa, Ont. | - - - |
|-----|--|-------|

Representing the Basic and Medical Sciences

- | | | |
|-----|--|----------------|
| 14. | Dr. R.L. de C.H. Saunders,
Department of Anatomy,
Dalhousie University,
Halifax, N.S. | 31 March, 1961 |
|-----|--|----------------|

* Member of Executive

Term to ExpireRepresenting the Basic and Medical Sciences (cont'd)

- | | | |
|-------|---|----------------|
| 15. | Dr. H.J. Barrie,
Department of Pathology,
University of Toronto,
Toronto 5, Ont. | 31 March, 1960 |
| * 16. | Dr. J.M.R. Beveridge,
Department of Biochemistry,
Queen's University,
Kingston, Ont. | 31 March, 1959 |
| 17. | Dr. J.B. Macdonald,
Director,
Forsyth Dental Infirmary for Children,
140 The Fenway,
Boston 15, Mass. | 31 March, 1960 |
| 18. | Dr. K.J. Paynter,
Division of Dental Research,
University of Toronto,
Toronto, Ont. | 31 March, 1960 |
| 19. | Dr. A. D'Iorio,
Department of Physiology,
University of Montreal,
Montreal, P.Q. | 31 March, 1961 |
| 20. | Dr. G.D. Scott,
Department of Physics,
University of Toronto,
Toronto, Ont. | 31 March, 1960 |
| 21. | Miss D.J. Wright, <u>Secretary</u> ,
National Research Council,
Ottawa 2, Ont. | - - - |

* Member of Executive

INITIAL DISTRIBUTION

(i) To members of the
Associate Committee on Dental Research

- | | |
|-----------------------------------|--|
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| 2. Brig. K.M. Baird | 14. Dr. K.J. Paynter |
| 3. Dr. H.J. Barrie | 15. Dr. A.G. Racey |
| 4. Dr. J.M.R. Beveridge | 16. Dr. R.L. de C.H. Saunders |
| 5. Dr. H.K. Brown | 17. Dr. G.D. Scott |
| 6. Dr. C. Castaldi | 18. Dr. E.W.R. Steacie |
| 7. Dr. A. D'Iorio | 19. Dr. D.L. Thomson |
| 8. Dr. R.F. Farquharson | 20. Dr. C.H.M. Williams |
| 9. Dr. C.D. Husband | 21. Miss D.J. Wright, <u>Secretary</u> |
| 10. Dr. L.A. Kilburn | |
| 11. Dr. J.-P. Lussier | |
| 12. Dr. J.B. Macdonald | |

(ii) To other persons:-

22-27 Deans of Dental Schools (who are not members of the Committee)

- | | | |
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| 22. Alberta | - | Dr. H.R. MacLean |
| 23. Dalhousie | - | Dr. J.D. McLean |
| 24. Manitoba | - | Dr. J.W. Neilson |
| 25. McGill | - | Dr. J. McCutcheon |
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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-SIXTH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

MARCH 3 - 4, 1960

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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Initial Distribution

By Invitation:

Dr. C. M. Davis

Regrets were received from:

Dr. D. L. Thomson and

Dr. R. F. Farguherson

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Twenty-sixth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

March 3 and 4, 1960

1. Minutes of the Twenty-fifth Meeting

The first session convened in the Council Chamber of the National Research Council, Ottawa, at 9:30 a.m., March 3, 1960.

1. Attendance:

Dr. K. J. Paynter, Chairman

Brig. K. M. Baird

Dr. H. J. Barrie

Dr. L. F. Belanger

Dr. C. Castaldi

Dr. M. A. Clay (for Dr. L. A. Kilburn)

Dr. A. D'Iorio

Dr. L. E. Francis

Dr. A. G. Gornall

Dr. I. Kleinberg

Dr. J. -P. Lussier

Dr. J. W. Neilson

Dr. J. B. Macdonald

Dr. J. B. Marshall

Dr. M. Poplove (for Dr. H. Brown)

Dr. R. L. deC. H. Saunders

Dr. G. D. Scott

Dr. C. H. M. Williams

Miss D. Wright, Secretary

By Invitation:

Dr. C. M. Dowse

Regrets were received from:

Dr. D. L. Thomson and

Dr. R. F. Farquharson

2. Introductory Remarks

The Chairman welcomed the new members of the Committee: Dean J. W. Neilson of the Faculty of Dentistry of the University of Manitoba, Dr. A. G. Gornall of the Department of Pathological Chemistry, University of Toronto, Dr. L. E. Francis of the Faculty of Dentistry, McGill University, Dr. L. F. Belanger, Department of Histology and Embryology, University of Ottawa, and Dr. I. Kleinberg, of the Department of Biochemistry, University of Manitoba. He also introduced Dr. C. M. Dowse, who had been invited to attend the meeting at the suggestion of Dean Neilson whose staff Dr. Dowse expects to join next session.

3. Minutes of the Twenty-fifth Meeting

The Minutes of the Twenty-fifth Meeting had been distributed to all members and were therefore taken as read.

The Secretary reported that an error had been made in item 10 (page 15) of the Minutes; 5 term grants totalling \$25,600 and 10 annual grants totalling \$28,300 were actually made from the 1959-60 allotment, rather than 4 term grants and 11 annual grants as shown in the summary.

It was MOVED by Dr. Lussier and SECONDED by Dr. D'Iorio THAT the Minutes be approved, as corrected.

CARRIED

4. Finances

The Chairman reported that the total funds available to the Committee for the year 1959-60 had been expended, except for that portion encumbered for the current meeting.

He noted that the Committee had requested an allocation of \$100,000 for the year 1960-61; he had been notified in January that it could expect to receive \$91,000 with which to carry out its programme.

On the basis of the requests received, the Chairman presented the following breakdown of possible expenditures for 1960-61:

Fellowships	\$ 36,000
Undergraduate Assistantships	6,500
Term grant commitments	25,600
Requests for annual grants	80,156
Requests for equipment grants	<u>9,415</u>

\$ 157,671

He reported that the President had been made aware of the Committee's position and had indicated that Council might give its approval to a revised allocation of \$101,000 if the requests for funds were considered sufficiently worthwhile. The Chairman pointed out that such an increased allotment was generous since it would mean that the Committee could hope to receive an increase of 25% in its allotment, or double that which could be made available to other Committees and Divisions of the Council for the coming year.

5. Chairman's Remarks

The Chairman then reviewed recent developments in Canadian dental research, the activities of the Associate Committee and the way in which it might best serve dental research in future.

(a) The Progress of Dental Research in Canada

"The Research Committee of the Canadian Dental Association last year conducted a follow-up survey of dental research in Canada, five years after an initial survey was conducted by the same Committee in 1954. It is evident from the data collected (see Appendix M) that a marked change in both the quantity and type of dental research being conducted in Canada has occurred: the quantity has increased, and the type has matured.

"All six of the dental schools in Canada now have research programmes under way, whereas five years ago only two were doing research. The amount of space available for research has increased eight fold. This has related to the school building programme which has been going on in Canada since 1954. Dalhousie has opened their new and much enlarged school, Toronto has moved into its new and expanded quarters, Manitoba has been provided with a new school, and building is going on in Alberta which will provide increased space for that school also.

"It is very significant that the number of dental research personnel who also hold basic science degrees has increased from some 37% of the total doing research in 1954, to 75% of the total in 1959. This indicates that the efforts of organizations (including this Committee) to encourage young graduates in dentistry to acquire basic science training have paid off handsomely.

"In addition, and what is even more important, is that this increase in research-trained personnel probably indicates a change in the type of research being conducted in dental schools. It implies an

increase in long-term basic investigations by career research workers and teachers, and a decrease in the type of short-term projects which were more popular five or six years ago. The former will ultimately pay much higher dividends in assisting in the understanding and prevention of dental diseases.

"It is good to see that the Universities are assuming their responsibility for supporting research in dental schools by not only providing the facilities, and carrying the overhead in relation to the facilities, but also by assuming the salaries of research personnel. This indicates a permanency for the dental research programme which would otherwise be lacking (e. g. were research workers paid by annual grants).

"In summary, it appears that from its beginnings a few years ago, dental research in Canada has grown rapidly and healthily. Nothing should be allowed to happen to discourage its growth.

(b) The Future of Dental Research in Canada

"There is no doubt that the expansion in dental teaching facilities, and therefore in dental research facilities, is going to continue for some time yet. Canada is still not training dentists in sufficient numbers to meet even to-day's needs, let alone to-morrow's. However even if no new schools were built within the next few years, the present schools are not yet fully staffed. All new staff, whether appointed as additions or replacements, should be trained in research methods.

"The great need for senior clinical personnel in dental schools to have research training is obvious in all the schools. Little if any research is going on to evaluate presently employed clinical methods, and the great wealth of material available in all school clinics for evaluation of treatment methods and procedures and for gathering epidemiological data relative to dental disease is largely wasted. It should be and could be utilized very successfully, were the men in charge of administering the clinic properly trained in research.

(c) Activities of the Associate Committee on Dental Research

"The Associate Committee on Dental Research was established in 1945 'to stimulate research in, or related to, the field of dentistry in Canada'. The Committee has done this by awarding funds allocated to it by the Council. Awards have taken several forms as follows:

"Grants-in-Aid: The Committee provides grants-in-aid of research to support projects by workers in the field of dental research. These may be either annual or term grants. Most of the awards have taken the form of annual grants in the past, although the Committee now is supporting five three-year term grants.

"Equipment Grants: The Committee is authorized to make non-recurring grants for equipment for the purchase of research equipment costing over \$5,000.

"Travel: By virtue of a change recently made in the general regulations governing grants, grantees are automatically authorized to spend \$100 per \$5,000 of grant or portion thereof each year for travel related to the project supported by the grant.

"Fellowships: The Committee also awards Graduate Dental Research Fellowships. Until last year these took two forms - Graduate Fellowships and Senior Fellowships. At the 1959 meeting it was moved that the classification of Senior Dental Research Fellowship be dropped, and that the upper limit of the Graduate Dental Research Fellowship be \$5,000 per annum. Dental Fellowships are not tenable with any other award. They are made to graduates in dentistry of high academic ability for the purpose of providing advanced training and experience in research in one of the basic sciences in dentistry. The Committee is presently supporting four Fellows.

"Summer Assistantships: Assistantships to support undergraduates in dentistry while working in a dental research laboratory during the summer months are also granted. The amount of these awards is based on the salary scale for payment of undergraduate university students working in the Council's own laboratories in Ottawa.

(d) The Role of the Associate Committee on Dental Research in the Future

"The success of the Committee in its efforts to 'stimulate research in or related to the field of dentistry in Canada' is reflected in the data of the C.D.A. survey. It also may be measured by comparing the amount of the original grant of \$35,000 made to the Committee in 1945 with our present allocation of \$101,000, or with what may be even more significant, with the amount of assistance requested this year.

"Grants-in-Aid: It is quite obvious that more money will have to be found to meet the increasing demand for grants-in-aid of research. This Committee is the sole body granting such support for dental research

on a national scale, and it should give serious consideration to ways and means of increasing its annual allocation. There is no point in working to stimulate research activities and to create new departments of dental research in the schools if, once founded, they cannot find support to continue their activities.

"There will be an increasing demand for term grants as research departments become more firmly established. The security offered by the term grant is something that will be sought by increasing numbers of research workers in dental schools in Canada.

"In addition, as the work of the research workers in each school becomes more integrated, there will probably be a demand for the block type of grant. This, of course, has the great advantage of permitting a more flexible programme and a greater integration of research efforts in an institution, than is possible under individual grants.

"Graduate Assistantships: There is also an increasing need to establish some sort of interim support for the young man or woman who has just completed his or her research training. This would permit such people to be employed on the staff of a dental school at a reasonable salary for a period of a few years at least, until they become settled. This type of grant would not only provide the young worker with an opportunity to become established in research before taking on too arduous a teaching load, but would also permit the administrators of the dental schools an opportunity to evaluate a prospective teacher before accepting him on the staff of the school in the relatively senior position his training would demand.

"Such an award is now being made in medical schools through Postgraduate Medical Research Assistantships. They are made by the Medical Committee as part of an annual, term, or block grant and are intended to support an individual for a period of not longer than six years.

"Research Associates: In addition, we should also consider providing for an award similar to the Medical Research Associate. This type of award provides full-time university appointment at the professorial rank to an individual who is highly qualified in research. The appointment is permanent, and carries with it all the advantages of a regular academic appointment in terms of tenure, etc. However, it is considered a research appointment and not a teaching appointment. A number of such awards have been made now through the Medical Committee with considerable success. The advantages of providing for such an appointment to dental schools would be great.

"Summer Assistantships: From comments recently received from the senior administrators of the dental schools across the country, it is obvious that the awards made by the Committee through the Summer Research Assistantships have been considered very valuable in guiding young dental students toward a career in research and teaching. It is too early yet, of course, to evaluate the long-term results of this programme, but its popularity is well established. There is good reason to assume that it will play an important part in providing Canadian dental schools with future research workers.

"Fellowships: I have received several comments lately from interested individuals concerning the amount of the annual stipend offered through Graduate Dental Research Fellowships. These are now as high as any of the Fellowships provided by the National Research Council, and higher than most in Medicine, and it would be unrealistic for us to raise them even further. It is of interest to observe that the Board of Trustees of the newly-established Queen Elizabeth II Fund for Research into Diseases of Children has set the amount of their Fellowships at \$4,000.

"I do not mean to imply that we should consider reducing the amount of the Dental Fellowships. We face a rather special problem in encouraging young graduates in dentistry to enter the research field. The financial sacrifice associated with such a move is considerably greater than it is for any other young university graduate. The young dentist can, even during his first year of practice, make a very good income, whereas for example, the graduate in medicine has at least one financially unproductive year of internship facing him. It is not possible, of course, to grant funds for Fellowships at the same level as a private practice income, but we should try to make the difference as little as possible.

"The problem of university fees for Fellows is also a serious one. One of our present Fellows, for example, out of his \$5,000 Fellowship must pay \$1,000 to the University in fees. It is not the policy of the National Research Council to provide funds for fees in addition to the Fellowship, particularly when the residency is in a Canadian university. Perhaps, however, some consideration might be given to the man who is studying in a University in a country other than Canada. This would be particularly pertinent to dentistry in as much as the opportunities for graduate study for dentists in Canada is not as great as it is, for example, in the United States where most of our Fellows train. As a matter of fact only one of the Canadian dental schools offers graduate instruction at all.

"The Committee should consider the possibility of permitting Fellows to earn a nominal salary in addition to their Fellowship. This is done in other areas by simply regulating that Fellowships are not tenable with any other major awards. The Medical Committee, for example, considers a major award to be something over \$500. I think it would be quite in order for us to alter our regulations to permit Fellows to do sufficient teaching or other work deemed useful by the sponsor, to earn an amount of up to \$500 in addition to his Fellowship."

6. Role of Other Government Agencies in Dental Research

The Chairman then invited the representatives of the three other Government agencies to acquaint the Committee with the current policies of their departments with respect to dental research.

Brig. Baird reported that the Royal Canadian Dental Corps does not undertake any actual research as such, although it does carry out some investigations related to dental supplies, equipment and materials, e.g. the use of stannous fluoride. He pointed out that the Defence Research Board is quite willing to sponsor research in military dentistry and would be glad to consider applications for grants-in-aid of research in this field. Brig. Baird offered to send the appropriate application forms to anyone interested in applying for funds from D. R. B.

Dr. Clay reported that there is no dental research done in the Department of Veterans Affairs in Ottawa, although there are a few men in the D. V. A. hospitals across the country who are interested in dental problems. Any research supported by D. V. A. must be carried out in its own hospitals; on occasion people are employed for the specific purpose of carrying out investigations of particular problems, but it is chiefly D. V. A. staff members who receive funds for research. He told the Committee that anyone who was interested in the D. V. A. programme could obtain further information from the office of Dr. L. A. Kilburn, Director of Dental Services, Department of Veterans Affairs.

Dr. Poplove reported that the Department of National Health and Welfare has funds for research relating to dental health procedures which might be applied on a mass basis. He cited studies on stannous fluoride and the Brantford fluoridation project. The research is usually carried out by members of the Department's Dental Health Division, although some support is also provided to investigators working in other institutions. The Department also supports individuals who are proceeding to the D. D. P. H. degree.

In reply to a question from Dean Neilson, Dr. Poplove said that there is no specific Dental Health Grant per se, as there is a Mental Health Grant, Crippled Children Grant etc., but funds for dental research can be provided from the General Public Health Grants which do exist.

Dr. Castaldi said that the dental schools may not be aware of the various sources of funds to which they might make application for research support. Dr. Macdonald suggested that it would be a useful undertaking for the Research Committee of the Canadian Dental Association to prepare a statement as to the sources of funds for dental research, which might be published in the C. D. A. Journal and brought to the attention of the dental schools.

The meeting was then opened to discussion of various points raised in the Chairman's remarks.

7. Graduate Assistantships

It was noted that under the general regulations governing National Research Council grants, it has always been possible for grantees to employ professionally trained people if sufficient funds were available to them; the Chairman asked for an expression of opinion as to the advisability of establishing a separate category of personnel support to provide for the salaries of young professionals apart from grants.

Dr. Macdonald felt that many research programmes would be greatly strengthened if grants could be large enough to enable a grantee to employ a biochemist or another specially qualified postdoctoral assistant. Dr. Gornall agreed that this would be highly desirable but he did not favour the establishment of a separate category of personnel support for such assistants; the funds should be made available to the grantee in his grant, if an assistant of this type is warranted. If a separate competitive category were established, the grantee would be greatly hampered because he would no longer be in a position to decide, without reference to the Committee, whether or not he could immediately employ a suitable person who might come to his notice.

No separate category of postgraduate assistants apart from grants was therefore deemed advisable at this time.

8. Research Associates

Dr. Belanger felt that if funds were available for a Dental Research Associateship programme, the universities would be in a much better position to encourage dental graduates to undertake advanced study in the basic sciences. At the present time most universities are not in a position to offer research professorships in the dental schools and dental research workers are therefore being attracted to institutions outside Canada.

The Chairman pointed out that Council had already authorized the Committee to appoint Dental Research Associates on terms similar to those governing Medical Research Associates, but to date none had been appointed. It was agreed that the implementation of this programme would add considerable impetus to dental research.

9. Fellowships

Dr. Macdonald reported that in the United States, Fellowships offered by the Public Health Service are much higher in value than any Canadian awards, but they create two problems: 1) many Fellows in training receive more than the junior members of the university staff who are training them, and 2) candidates are being attracted to the training programme merely by the generosity of the stipend; both situations were undesirable. He therefore agreed with the Chairman that the Fellowship stipend should not be raised at the present time. However, after discussion of the matter of fees payable by Fellows, it was MOVED by Dr. Castaldi and SECONDED by Dr. Macdonald

THAT, with the approval of his supervisor, a Fellow may be permitted to carry out teaching duties not to exceed four hours per week, for which he may accept payment in an amount up to the equivalent of his tuition fees.

CARRIED

10. Site of Meeting

The Chairman reported that Dean Neilson had extended an invitation to the Committee to hold its next meeting in Winnipeg. He noted that meetings have normally been held in Ottawa because this eliminates the necessity of transporting all the materials and records needed at the meeting to another place. However, these administrative difficulties could probably be overcome if it were felt that holding a meeting elsewhere

would stimulate dental research in the particular location of the meeting.

It was MOVED by Dr. Macdonald and SECONDED by Dr. Saunders

THAT the Secretary be instructed to thank Dean Neilson for his kind invitation to meet in Winnipeg and that the decision as to the feasibility of accepting be determined on an administrative basis.

CARRIED

Later in the meeting, it was decided with regret that Dean Neilson's invitation must be declined; the additional funds it would entail were, instead, allocated to the Committee's 1960-61 grants programme.

11. Closing Date for Receipt of Grant Applications

The Chairman reported that during the last two years he had been given the opportunity to sit with an informal Committee called the Inter-departmental Medical Research Co-ordinating Group which has met in Ottawa in mid-January both this year and last, to discuss matters pertaining to the granting of funds to support medical research in Canada. Representatives of several of the larger Canadian Government and voluntary organizations set up to administer funds to support medical research on a national scale were present at the meeting. This Group has agreed to meet again next year at approximately the same time, i.e. about mid-January. It would be of benefit to its members if a list of the applications for funds received by the Dental Committee were available for their meeting. To make such a list available would require that the date for receiving applications for Grants-in-Aid of dental research be moved up by at least one month to January 1st.

It was MOVED by Dr. Lussier and SECONDED by Dr. Kleinberg

THAT the closing date for the receipt of applications for Grants-in-Aid of dental research be January 1st.

CARRIED

12. Grantees' Progress Reports, 1959-60

Reports on work carried out during 1959-60 were received from ten investigators holding annual grants and two who had been awarded term grants. Copies of all reports had been distributed to the members

for review prior to the meeting, and summaries were presented at the meeting either by the grantee himself or a member of the Committee designated by the Chairman.

- (a) Project DA-75. C. Castaldi - "A study of enamel faults and its relationship to the time of cerebral damage" (see Appendix A); presented by himself.
- (b) Project DA-76. L.E. Francis - "Studies on gingival changes due to drug administration" (see Appendix B); presented by himself.
- (c) Project DA-77. H.W. Hart - "Dental health aspects of Arctic natives" (see Appendix C); presented by Dean Neilson.
- (d) Project DA-74. A.E. Hoffman - "The use of an anorganic bone graft following partial mandibular resection in the rat" (see Appendix D); presented by Dr. Saunders.

In connection with the work of Dr. Hoffman, Dr. Williams suggested that he might find the work of W.G. Cross of Guy's Hospital of interest.

- (e) Project DA-78. I. Kleinberg - "Further studies on the role of the dental plaque in the caries process; further studies on the effect of substances removed during the refining of carbohydrates on calcium phosphate solubility" (see Appendix E); presented by himself.
- (f) Project DA-23. C.P. Leblond - "Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements" (see Appendix F); presented by Dr. Belanger.
- (g) Project DA-71. E.M. Madlener - "The characterization of an experimental 'Fusospirochetal' lesion" (see Appendix G); presented by Dr. Macdonald.

Dr. Macdonald reported that, following the instructions of the Committee at its last meeting, he had written to Dr. Madlener suggesting ways by which he could reach his objective and thus terminate the project during the year. It was noted that an application for further support for this project had been received.

- (h) Project DA-79. Z. Mezl - "Penetration of minerals from saliva and shifting of minerals in the carious lesion" (see Appendix H); presented by Dr. D'Iorio.

It was felt that Dr. Mezl's report lacked sufficient details as to his methods and results; Dr. Lussier agreed to advise him to present a more informative report on his future work.

- (i) Project DA-64. K. J. Paynter - "Investigations into the nature of cementum" (see Appendix I); presented by himself.
- (j) Project DA-80. J.G. Perreault and W.B. Donohue - "The effects of a heavy dose of roentgen rays on the developing structures of the skull in young kittens" (see Appendix J); presented by Dr. Barrie.
- (k) Project DT-61. A.S.V. Burgen - "Secretion of bicarbonate ions in the saliva; changes in the secretion of the developing salivary gland" (see Appendix K); presented by Dr. Kleinberg.
- (l) Project DT-70. J. -P. Lussier - "Hormonal factors and their relationships of citrate metabolism in bones and teeth" (see Appendix L); presented by himself.

13. Regulations for the Treatment of Experimental Animals

In reviewing the work done under grant DA-80, Dr. Barrie opened discussion of the broad question of the treatment of experimental animals. Since research is more and more being equated with the use of animals, he felt that there is a growing need for the proper appreciation, by investigators, of the fact that in animal experimentation they are dealing with living tissue. Every care should therefore be taken, first to determine whether the procedures carried out on animals will actually make a useful contribution to our knowledge of any particular subject and, second, to ensure that experimental animals are well treated during the course of any such procedures which are deemed necessary. He felt that the Committee had a definite responsibility for seeing that these precautions were taken in any projects which it supports. Unlike Great Britain and other countries, Canada does not yet have official regulations

regarding the use of animals for experimental purposes; rather than allowing matters to reach the stage where public concern made legislative intervention necessary, Dr. Barrie felt that scientists themselves should take the initiative in drawing up a code for the proper use of animals in experimentation and the proper treatment given them during the course of the research.

All members of the Committee agreed with the principles outlined by Dr. Barrie. Dr. Saunders suggested that an effort be made to learn more about the regulations enforced in other countries. Dr. Macdonald said that the British regulations are contained in a manual of animal care, dealing with all types of large and small laboratory animals, put out by the Medical Research Council. Dr. Neilson added that the Animal Welfare Institute in New York also issues literature on the subject.

Dr. Barrie suggested that the Committee forward to Council a statement of its concern about the treatment of experimental animals and a request that steps be taken to improve the situation. Dr. Gornall, however, felt that since the laboratories of the Council itself do not carry out much animal work, it might be more appropriate to bring the question to the attention of the Canadian Federation of Biological Societies. It was AGREED that the Chairman should write to the Honorary Secretary of the Federation, Dr. E. H. Bensley, recommending that the Federation undertake the preparation of a code for the handling of experimental animals which might be effected in Canada.

Dr. Lussier agreed to pass on to Drs. Perreault and Donohue the Committee's feeling regarding the experiments on which they had been engaged.

14. Publications of Grantees

The Chairman reported that at his request the Secretary had written to all those who had held grants from the Committee during the period 1954 to 1959, asking for a list of publications arising out of work so supported. On the basis of the replies received, a list of 77 papers had been compiled (see Appendix N). It was AGREED that a similar list should be compiled every three or four years.

15. Meeting of the Executive

The Chairman reported that the Executive had met on the evening of March 3rd to give preliminary consideration to applications

for Fellowships, Grants-in-aid and Summer Assistantships. The recommendation of the Executive was submitted to the Committee for approval or modification, as each application was reviewed.

16. Applications for Fellowships, 1960-61

A total of 8 applications were considered: 4 for the renewal of current Fellowships and 4 new applications. The following action was recommended:

Blackmore, R. V.	To work in the Dept. of Bacteriology, Strong Memorial Hospital, University of Rochester, under Dr. H. R. Morgan (renewal)	APPROVED \$4,500
Dale, J. G.	To work in the Dept. of Histology, Forsyth Infirmary, and Harvard School of Dental Medicine, under Dr. J. T. Irving (renewal)	APPROVED \$4,500
Demircioglu, A.	To work in the Division of Dental Research and Dept. of Anatomy, University of Toronto, under Dr. K. J. Paynter	APPROVED \$3,500
Johnston, M. C.	To work in the University of Rochester School of Medicine and Dentistry, Dept. of Dentistry and Dental Research under Dr. E. Johansen (renewal)	APPROVED \$5,000
Listgarten, M. A.	To work in the Harvard School of Dental Medicine, under Drs. Sognnaes and Goldhaber	APPROVED \$3,000
Socransky, S. S.	To work in the Harvard School of Dental Medicine and Forsyth Infirmary, under Dr. J. B. Macdonald (renewal)	APPROVED \$4,500

Wasylichuk, M. To work in the Dept. of Biochemistry, University of Alberta, under Dr. E. T. Pritchard

APPROVED \$2,500

Winnick, A.N. To work in the Forsyth Dental Infirmary

NOT APPROVED

The total allocated to Fellowships for 1960-61 was therefore \$27,500.

17. Applications for Grants, 1960-61

The Chairman reported that the Committee already had commitments of \$25,600 for the coming year, for five term grants: Dr. L. F. Belanger (DT-52, \$4,500), Dr. A.S.V. Burgen (DT-61, \$6,000), Dr. D.H. Copp (DT-59, \$5,300), Dr. J.-P. Lussier (DT-70, \$4,800) and Dr. K. J. Paynter (DT-72, \$5,000).

Nineteen applications for operating and two for equipment grants were then considered. Since the amount of applications received by the Committee was far in excess of the funds available, it was necessary to reject nine applications completely and cut nine more; only two could be granted in the amount requested, and one additional application, while approved in principle, was deferred for payment if funds became available.

<u>Applicant</u>	<u>Title & Amount Requested</u>	<u>Recommendation</u>
Baril, C., Orthodontics, Montreal	Electromyography of muscles innervated by the VIIth cranial nerve \$4,000	NOT APPROVED
Baril, C., Orthodontics, Montreal	To purchase one electroencephalograph, Grass, model IVB, with accessories \$5,800	APPROVED \$5,800
Chagnon, M., Psychology and Education, Ottawa	Psychological adjustment and adolescent oral health \$3,050	NOT APPROVED

<u>Applicant</u>	<u>Title & Amount Requested</u>	<u>Recommendation</u>
Dowse, C. M., Dentistry, Manitoba	Metabolism of periosteum \$5,440	APPROVED \$3,500
Feinchneider, I., Dental Surgery, Montreal	1. Pulp therapy with anti- biotics 2. Intra-osseous implants \$2,400	NOT APPROVED
Findlay, J., Periodontics, Dalhousie	An investigation into the effects of an artificially in- duced Oestrus cycle on the periodontal tissues of hypo- physectomised rats \$1,677	NOT APPROVED
Francis, L. E., Pharmacology, McGill	Effects of drug administration on gingival histamine and serotonin DT-76 \$6,000	APPROVED three-year TERM grant of \$5,000
Hart, H. W., Prosthetic Dentistry, Manitoba	Dental health aspects of Arctic natives DA-77 \$1,200	NOT APPROVED
Hoffman, A. E., Oral Surgery, Dalhousie	The use of an anorganic bone graft following partial mand- ibular resection in the rat DA-74 \$4,150	APPROVED \$2,500
Hunt, A. M., Dentistry, Toronto	Measurement of strontium ⁹⁰ and carbon ¹⁴ in primary teeth of Canadian children \$6,200	APPROVED \$4,500
Kleinberg, I., Biochemistry, Manitoba	1. Further studies on the role of the dental plaque in the caries process. 2. Further studies on the effect of substances removed during the refining of carbohydrates on calcium phosphate solubility DA-78 \$5,959	APPROVED \$5,500

<u>Applicant</u>	<u>Title & Amount Requested</u>	<u>Recommendation</u>
Leblond, C. P., Anatomy, McGill	Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements DA-23 \$5,000	APPROVED \$4,000
Lussier, J. -P., Dental Surgery, Montreal	To purchase electrometer and accessories \$3,615	APPROVED in principle, but deferred for payment if funds become available
Madlener, E. M., Dentistry, Toronto	The characterization of an experimental 'Fusospirochetal' lesion DA-71 \$4,200	NOT APPROVED
Marshall, F. J., Oral Histology & Endodontology, Manitoba	In vivo studies of dentine permeability and root canal filling seals with radiosulfur (S ³⁵) \$3,500	APPROVED \$2,200
McCallum, J. L., Medicine, Montreal General Hospital	Further studies on the etiology of aphthous lesions of the mouth \$5,000	NOT APPROVED
Mezl, Z., Dental Surgery, Montreal	Penetration of minerals from saliva and shifting of minerals in the carious lesion DA-79 \$800	APPROVED \$800
Nikiforuk, N., Dentistry, Toronto	Organic compounds of dental tissues \$5,300	APPROVED three-year TERM grant of \$5,000
Perreault, J. G. Pediatric Dent., Donohue, W. B., Diagnosis, Montreal	The effects of a heavy dose of roentgen rays on the develop- ing structures of the skull in young cats DA-80 \$4,740	NOT APPROVED

<u>Applicant</u>	<u>Title & Amount Requested</u>	<u>Recommendation</u>
Trott, J.R., Dentistry, Manitoba	An investigation into the development of the epithelial attachment, and the reaction of the gingivae to varying irritants, both local and systemic \$6,800	ENCUMBERED \$3,000 to be released by the Executive following receipt of clearer research proposal
Youdelis, W.V., Mining and Metallurgy, Alberta	Fundamentals of amalgam metallurgy \$4,740	NOT APPROVED

Summary of grants:

21 applications received, totalling	\$89,571
11 grants recommended, totalling	41,800
1 grant deferred, totalling	3,615

18. Undergraduate Summer Assistantships

Consideration was then given to applications for Summer Dental Research Assistantships submitted to the Committee by dental undergraduates seeking employment in the laboratories of grantees of the Committee. A total of 16 applications were received: 5 from McGill, 2 from Montreal, 4 from Toronto, 1 from Dalhousie and 4 from Manitoba (see Appendix O).

Awards were recommended as follows:

Berube, M.	To work in the laboratory of Dr. J.-P. Lussier at Montreal	3 mos. @ \$285: \$855
Direnfeld, V.N.	To work in the laboratory of Dr. K.J. Paynter at Toronto	3 mos. @ \$265: \$795
Fletcher, R.G.	To work in the laboratory of Dr. C.P. Leblond at McGill	3 mos. @ \$305: \$915
Jow, R.	To work in the laboratory of Dr. A.M. Hunt at Toronto	3 mos. @ \$305: \$915

Trester, P. H.	To work in the laboratory of	3 mos. @ \$285:
	Dr. I. Kleinberg at Manitoba	\$855

An Assistantship was also approved for Mr. G. H. Weinlander to work in Dr. Leblond's laboratory from June 1st to August 15th. Dr. Belanger, who had indicated his willingness earlier to employ a dental undergraduate in his laboratory but had been unable to obtain one because the University of Ottawa has no dental school, volunteered to transfer the sum of \$760 from the 1960-61 instalment of his term grant DT-52 to Dr. Leblond's grant DA-23 to permit Mr. Weinlander to hold an Assistantship at the rate of \$305 per month for 2-1/2 months. This generous offer was accepted by the Committee.

The following applicants were also considered suitable candidates for Assistantships:

Abood, J. G., to work with Dr. A. S. V. Burgen at McGill;
Booth, W. G., to work with Dr. C. Leeson at Dalhousie, and
Kaufman, H. W., to work with Dr. I. Kleinberg at Manitoba.

Since the funds available did not permit their employment under the Committee's auspices, it was hoped that the Research Committee of the Canadian Dental Association would give favourable consideration to supporting these students at the same rate as provided by the Associate Committee.

Dr. Francis suggested that the announcement sent to the dental schools be made more specific. He felt that each applicant for such employment should consult the head of the department in which he hoped to work during the summer, to ensure that he would be accommodated if his application to the Committee were successful. Some members of the Committee reported that it was their practice to have all applications given a preliminary screening by members of the Dental Faculty and only those who could be accommodated were encouraged to apply to the Committee. This, however, was not feasible for other schools.

It was agreed that, in future, the announcements would request applicants to approach a specific member of the Associate Committee at their particular school rather than writing directly to the Committee's Secretary. Applications could then be screened in all dental schools, with only the best being forwarded to the Secretary for presentation at the meeting.

It was MOVED by Dr. Francis and SECONDED by Dr. Belanger
THAT it be recommended to Council that the appointments
of Dr. Castaldi and Dr. Scott be renewed for a further
period of three years.

CARRIED

Dr. Neilson enquired about the academic level at which Assistantships were most likely to be provided by the Committee. The Chairman said that it had been the consensus of opinion that a first- or second-year student would normally receive preference over a third-year student for a first Assistantship. A third-year student has no opportunity for holding a renewal of an Assistantship and it is to the advantage of both the student and the supervisor for the training to be provided for at least two summers.

19. Summary of Action Regarding Applications

The Committee therefore allocated funds as follows:

7 Fellowships	\$27,500
7 Term Grants	35,600
8 Operating (Annual)	26,000
1 Equipment Grant	5,800
5 Summer Assistantships	4,335
Committee expenses	<u>2,000</u>

\$101,235

+ 1 Equipment grant of \$3,615 approved in principle but deferred pending availability of funds.

It was MOVED by Dr. Williams and SECONDED by Dr. Saunders

THAT the above expenditures be recommended to Council.

CARRIED

20. Membership of the Committee

The Chairman informed the Committee that the term of appointment of six members would expire on March 31, 1960: Dr. Castaldi, Dr. Scott, Dr. Barrie, Dr. Lussier, Dr. Macdonald and Dr. Williams. According to the regulations governing Committee membership, no member may serve for more than two consecutive terms; the last four named were in this category.

It was MOVED by Dr. Francis and SECONDED by Dr. Belanger THAT it be recommended to Council that the appointments of Dr. Castaldi and Dr. Scott be renewed for a further period of three years.

CARRIED

Nominations were then requested for new members to replace the retiring members who were ineligible for re-appointment. After due consideration of various nominees, in relation to fields of interest and geographical representation, it was AGREED that the following be recommended to Council for three year appointments to the Committee: Dr. A. J. Rhodes, School of Hygiene, University of Toronto; Dr. G. Nikiforuk, Division of Dental Research, University of Toronto; Dr. C.P. Leblond, Department of Anatomy, McGill University, and Dr. R. J. Slater, Research Institute, Hospital for Sick Children, Toronto.

Dr. P.S. Christie of Halifax and Dr. D. Wagner of Ottawa were agreed upon as alternates.

On behalf of the Committee the Chairman thanked the retiring members for the interest they had taken in furthering the aims of the Committee, for their valuable contributions at its meetings and their work on and with the Executive during the past six years.

21. Executive for 1960-61

Members of the Executive for 1959-60 had been the Chairman, Dr. Lussier, Dr. D'Iorio, Dr. Williams and Dr. Scott. Since Dr. Lussier and Dr. Williams were retiring from the Committee, it was AGREED that Dr. Kleinberg and Dr. Gornall would replace them on the Executive. The other members of the current Executive agreed to continue to serve during 1960-61.

22. Estimates for 1961-62

It was the general feeling of the Committee that dental research in Canada would expand rapidly in the next few years. The Chairman remarked that although the Committee has, since its establishment, received proportional increases, it was now apparent that with the development of new schools, the increased physical facilities in the older ones and the interest in dental research being aroused among outstanding graduates, research programmes have grown faster than the funds available for their support. He felt that the time had come to approach Council for an immediate substantial increase to which a percentage increase might be added in later years.

Dr. Marshall suggested that an effort be made to determine the number of people being trained in dental research and the extent to which they can be absorbed in the Canadian dental schools in the next few years.

If the number and type of appointments could be ascertained, the amount of money required to support research in four to five years might be calculated on the basis of five to ten thousand dollars for each person doing research.

Dr. Gornall cautioned against basing the requirements of men entering research on the amounts provided for dental research workers in the past. Much of the research in years gone by was done by dental practitioners on a part-time basis, whereas dental research is being done to an increasing extent by men trained in the basic sciences; the research is more mature, more intensive and requires more funds.

Dr. Williams suggested that in view of the increased interest in postgraduate training on the part of dental graduates, as evidenced by the increase in applications for dental fellowships submitted to the Committee, one might reasonably count on at least four trained people per year becoming available for staff appointments in the dental schools. This would almost double the number of investigators in five years. The availability of Dental Research Associateships would also contribute to an increase in the number of dental graduates entering research careers.

Dr. Macdonald felt that the availability of adequate funds would do much to stimulate dental research in Canada; it would enable research investigators to make a realistic appraisal of the support needed to maintain their research on a sound basis. He added that the work of the established investigators now supported by the Committee merited twice the support that could be provided and, in their hands, additional funds would have been used to advantage.

Dr. Kleinberg agreed that many applicants are aware of the benefits which would accrue to their research if they were in a position to obtain the services of highly qualified assistants; knowing that the necessary funds have not been available for this purpose, however, they have not included such items in their applications.

Therefore, on the basis of the need for a more realistic approach to the costs of dental research, the need for professional assistance in carrying out the research, the desirability of initiating the Dental Research Associateship programme, and the expansion of research which can be expected as a result of the 8-fold increase in available space, it was MOVED by Dr. Macdonald and SECONDED by Dr. Castaldi

THAT the allocation of the Associate Committee on Dental Research for 1961-62 be \$200,000.

After discussion of the motion and further consideration of the increase in applications for personnel and project support which could be anticipated,

an AMENDMENT was proposed by Dr. Kleinberg and
SECONDED by Dr. Gornall

THAT the budget of the Associate Committee on Dental Research be raised to \$225,000 for 1961-62.

Motion as amended, CARRIED

It was further MOVED by Dr. Macdonald and SECONDED by
Dr. Neilson

THAT the Chairman be instructed to work with the Executive to prepare complete and careful documentation to support the request for a budget of \$225,000.

CARRIED

23. Fluoridation

Dr. Castaldi enquired as to whether the National Research Council had taken an official stand on the question of the fluoridation of communal water supplies, as had the National Research Council of the United States. He felt that this would contribute greatly to the settlement of a question that is currently causing a great deal of concern in many areas of the country.

The Chairman replied that matters such as this, in Canada, are not within the scope of the National Research Council but fall within the jurisdiction of the Department of National Health and Welfare.

The meeting adjourned at noon on March 4th.

APPENDIX A

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Subject: A study of enamel and dentine hypoplasia in humans with cerebral palsy.

Grantee: C. R. Castaldi

Project: D.A. 75

Amount Awarded: \$2,385.00 - 1959-60

This study is divided into two parts:

- (a) clinical
- (b) histological examination of collected teeth.

The clinical part of this study has been supported by a grant from the Department of National Health and Welfare and consists of examination of children with cerebral palsy by the following team:

Pediatrician and orthopedic surgeon who examine the children and establish the diagnosis with specific reference to the type of cerebral palsy.

Obstetrician who reviews the mother's prenatal, neonatal and postnatal history by means of interviews with the parents and review of hospital records.

Geneticist who studies the dermal patterns of fingers, palms and soles of the children.

Dentist who examines the mouths of the children and determines the incidence of enamel hypoplasia and the frequency of these defects on the various surfaces of the teeth.

As the teeth are shed or lost prematurely due to caries they are collected, ground sections are made and these are studied for occurrence of pre- neo- and postnatal quality of enamel. These findings are then compared with the clinical dental examination findings, the medical diagnosis, the dermal patterns and the mother's prenatal and neonatal history.

The primary purpose of this study is to determine whether certain types of cerebral palsy are prenatal in origin. If so, such natal factors such as prematurity, difficult delivery or anoxia would in fact be incidental and not causal. Also, if one type was

shown to be of prenatal origin then, it would be possible to concentrate on this type in an endeavour to find out the specific causal factors occurring in the prenatal period.

To date 175 children have been under study. Breakdown into diagnostic groups is as follows:

Symmetrical spastic diplegia	- 41
Asymmetrical spastic quadriplegia	- 29
Spastic hemiplegia	- 74
Athetoid quadriplegia	- 31
	175

Of these, 148 have had genetic studies done and 130 have had clinical dental examinations. Eighteen of the 130 were too young to be adequately evaluated for hypoplasia of enamel because all primary teeth have not yet erupted but are still being followed for further examination; 24 had such severe dental caries, evaluation was not possible and a total of 11 cases of triplegia, ataxia or mixed types of cerebral palsy have been excluded because of insufficient numbers.

DERMAL PATTERNS¹

Normal Population	30% mixed (or overlap) pattern 70% normal pattern
Mongoloids	70% mongoloid pattern 30% overlap pattern

Dermal Patterns

Only in symmetrical spastic diplegia was there a significant difference in the dermal patterns found as compared to what might be expected in "normal" children. There were 40.5% normal or mature patterns and 59.5% overlap or mixed patterns.

Clinical Dental Examination

Diagnosis of enamel hypoplasia was made in the following way. The teeth were first cleaned with a toothbrush. If there was evidence of a visible pit (excluding carious areas and normal anatomic pits) or a series of pits or grooves arranged horizontally on the surface of the tooth and if the defect could be easily felt as a definite depression when the examiner ran his finger nail over

the area in question the patient was said to have enamel hypoplasia.

Of 77 cases adequate for evaluation enamel hypoplasia occurred as follows:

Enamel Hypoplasia by Diagnostic Groups
(77 Cases)

<u>DIAGNOSIS</u>	<u>ENAMEL DEFECTS</u>	<u>NO DEFECTS</u>	<u>BIRTH HISTORY OF THOSE WITH DEFECTS</u>
Diplegia	15	9	12 Premature 0 Kernikterus
Quadriplegia	2	12	1 Premature 1 Kernikterus
Hemiplegia	2	29	1 Premature 1 Kernikterus
Athetoid	7	1	1 Premature 6 Kernikterus

The hypoplastic defects have occurred more often on the labial surface than on the lingual surface of the teeth. In the control series which we are presently running using well first grade school children, the hypoplastic defects have occurred in approximately 12% of the cases as compared to 31% of the cerebral palsy cases. The defects have occurred more often on the labial than on the lingual surface in the control series.

The histological work has been considerably delayed because the tooth cutting machine ordered last spring did not arrive until November. To date too small a number have been sectioned to be reported on. The method consists of making tracings from photographic enlargements of the ground sections, determining whether the striations found are in prenatal, neonatal or postnatal positions on the teeth and then comparing these finds with clinical findings.

¹ Walker, N. F. Pediatric clinics of North America; 531-545.
May 1958.

APPENDIX B

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH NATIONAL RESEARCH COUNCIL

January 1960

Subject: Studies on gingival changes due to drug administration.

Grantee: L. E. Francis

Project: D.A. 76 Amount Awarded: \$5,100.00 - 1959-60

With the help of Mrs. B. Koss Levine and using the previously discussed methods, studies have continued on the effects of diphenylhydantoin and other substances on the histamine and serotonin content of human and animal gingival tissue. The results of some of these experiments have been published by Dr. K. I. Melville and myself in the Canadian Dental Association Journal, Vol. 25, No. 10, Oct. 1959, and also in the Journal of Dental Medicine, Vol. 14, No. 4, Oct. 1959.

The summary of the experiments up to that time were as follows:

- (1) That neither Mesantoin nor Phenobarbital administration lead to any significant increase in extractable gingival histamine. While in the dilantin-fed dog gingiva there appeared to be a decided increase in comparison with the normal gingival tissue which shows evidence of some histamine antagonizing or anti-histamine substance.
- (2) It is apparent that diphenylhydantoin administration does not alter extractable histamine content of lung tissue. However extracts of the ileum also showed histamine antagonizing action.
- (3) Extracts of normal gingival tissue of the dog contain relatively high quantities of extractable serotonin, and following prolonged dilantin administration little or no evidence of serotonin is detectable in the extracts. This latter effect is also associated with development of some temporary antagonism to the subsequent responses to serotonin.
- (4) It is concluded that both diphenylhydantoin and serotonin lead to a significant increase in brain serotonin.
- (5) It is evident that human gingiva contains only relatively small quantities of demonstrable histamine, but all extracts showed marked "histamine-blocking" actions. However, it is clear that this tissue is rich in serotonin.
- (6) It would appear that in inflamed gingival tissue there is a high histamine content but little or no demonstrable serotonin.

(7) It also appears that extracts obtained from the hyperplastic gingiva, excised from a patient previously treated with dilantin and phenobarbital, shows relatively little histamine and complete absence of serotonin.

Since that time 52 extracts from human gingiva have been examined to establish what might be considered to be normal amounts of histamine and serotonin, and to have a better understanding of the alterations. These figures were found to be 8.3 - 33 micrograms/gram tissue for histamine and 30 - 90 nanograms/gram tissue for serotonin. Since it has been considered that the altered histamine and serotonin may be the "trigger mechanism" responsible for the production of hyperplastic tissue, substances such as l-tryptson and pyridoxine which are chemical precursors of 5-Hydroxytryptamine (serotonin) are being used in an attempt to increase the serotonin of the gingiva. Pyridoxine has been used to increase the brain serotonin of the ferret. Using the drug itself the serotonin level of the brain was raised from normal level of 146 nanograms/gram tissue to 209 nanograms/gram tissue, and combined with dilantin up to 355 nanograms/gram tissue, and with mesantoin up to 279 nanograms/gram tissue. In progress an attempt is being made to precipitate gingival hyperplasia in dogs by depleting them of the gingival serotonin with the use of desoxypyridoxine which destroys pyridoxine and therefore possibly inhibits the production of serotonin.

It was also of interest to attempt the isolation of the histamine antagonist that occurs in normal human and dog tissue. This has been started in collaboration with Dr. Donald Douglas, of the Montreal General Hospital, and Dept. of Metabolism. Dr. Douglas has set apart a section of his laboratory for this study. This aspect of the program has just begun.

Separation of the histamine-antagonizing like substance has been attempted by column chromatography with activated charcoal and other suitable adsorbants, and by paper chromatography. Isolation of the inhibitory factor will be followed by characterization of physical and chemical means. Since the data to date are too incomplete to warrant definite conclusions all experiments are being continued with the hope that studies in relation to therapeutics may be carried out in the near future.

APPENDIX C

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Subject: Dental health aspects of Arctic natives.

Grantee: H. W. Hart

Project: D.A. 77

Amount Awarded: \$1,650.00 - 1959-60

Purpose

This project was set up to investigate dental and associated oral conditions of a "breed isolate" group of Arctic Indians on whom considerable general medical data had already been reported. The group is the Vanta Kutchin of the Loucheux tribe; these natives dwell in their native village of Old Crow in the far north west corner of the Yukon Territories, some one hundred miles inland from the Arctic coast.

The purpose of the investigation was twofold: one phase, somewhat anthropological in nature, concerned jaw formation and development, genetics of the teeth and any resultant morphological characteristics; the second phase, therapeutically slanted, was to concern relief of pain and other treatment of an emergent or essential nature for the native populace.

The project was carried out during the month of July in two full weeks of active work on location; one intended work week was lost at the outset due to inclement weather enroute, when it was impossible to proceed via air from Aklavik, N. W. T. to the Old Crow site.

Procedure and General Findings

Of the total populace of approximately 160 natives, over 100 adults and children over the age of six years were examined and charted dentally. Also, many of the children six years of age and under were checked orally.

It became immediately and forcefully obvious what this native group needed was not so much investigation, but treatment - and treatment in far greater measure than the planned project could possibly allow. From the standpoint of humanity, this fact demanded recognition and action; to continue, in the human vein - necessity dictated that this worker's time be divided, as it actually was by count, in the ratio of 90% examination and treatment to 10% investigation.

In respect to dental caries, the more mature segment of the population - say, from 35 years of age upwards - exhibited almost negligible effect from this scourge, with decreasing past and present evidence of onset as rank in years progressed; contrarily, in considering the younger adults, youth groups and the immature, the increase of onset was remarkably apparent diminution in age. A marked dietary trend in recent years at the village suggests itself in association with the above factors. What conclusions there are to be drawn from the evidence noted will be attempted in the forthcoming complete report.

Jaw structure, both complete as well as in active stages of development, seemed excellent. Tooth development and morphology, as noted from the mature fine dentitions of the more senior adults, followed normal patterns; those certain few dentitions in the younger generations which were noted to be relatively caries-free seem to hint that a dominant genetic pattern or influence, could all other factors be equalized, might prevail.

The supporting structures of the teeth in all age groups evidenced remarkable general health. Even though oral hygiene as it is commonly understood is not at all well practised - the teeth themselves often being badly stained, encrusted and uncared for - nevertheless only a minimal marginal gingivitis was usually apparent. The gingivae remained intact, were usually not hemorrhagic, and generally indicated they enjoyed a highly resistant state. Pocket formation even in the aged was minimal; few of the many teeth extracted were removed because they were loose or periodontally sore. Spot radiographic checks of bone levels and pattern support the above favorable outlook.

Models of suitable dental arches and dentitions, representing about one third of the populace examined, were made for the permanent record and further comparative study. Jaw angle measurements were crudely taken of the entire group and added to the record for future interest; a few jaw angles were also recorded radiographically.

Observation of the throat areas and the oral cavity in its entirety is worthy of note here, revealing consistent general features for the whole population as follows: the oral mucous membrane, gingivae, tongue and throat were almost invariably clean, free of plaques, glistening in health, uniformly and lightly moist. It was of considerable interest to note at the time there was not a coated tongue in the populace. Furthermore, saliva was seldom found to be thick, ropery, stringy or of heavily viscous consistency; in all mouths it seemed close to the easily flowing, properly viscous ideal.

Recommendations and Conclusions

From the work to date, it is recommended that the project be continued. Possibly a further combined endeavour, by the three agencies involved so far, could be arranged in such a way that an additional study project along with treatment might be undertaken. Such a conclusion will be drawn in a further report on the project.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Subject: The use of an anorganic bone graft following partial mandibular resection in the rat.

Grantee: A. E. Hoffman

Project: D.A. 74 Amount Awarded: \$2,350.00 - 1959-60

The awarding of a grant by the National Research Council for project D.A. 74, initiated the establishment of a research laboratory in the Faculty of Dentistry, Dalhousie University. As a result, a portion of the past year has been devoted to the procurement of the necessary equipment and organization of our facilities. Through the co-operation of the Research Division of Armour and Company of Chicago, Illinois, a quantity of "ossar" (anorganic bone) was made available.

Preparation

The anorganic bone was shaped so as to be of suitable dimensions for the contemplated mandibular defect and autoclaved just prior to the surgical procedure. Twenty-four hours prior to surgery the rats were placed on a prepared liquid diet consisting of Purina Fox Chow, Paramette syrup and antibiotics (Ilotycin). One hour pre-operatively the rats were administered a suitable dose of atropine sulphate and occasionally sodium nembutal intraperitoneally.

Procedure

Under a general anesthetic consisting of ethyl ether, administered by the open drop method, the surgical area was adequately prepared and approximately 1 c.c. of 2% Xylocaine containing epinephrine 1/50,000 was injected into the region to aid in the procurement of hemostasis.

The mandible was exposed via a submandibular approach and resection of a portion of the mandible performed. Usually a 3 - 5 m.m. segment of bone was removed. The anorganic bone graft was then inserted and secured in position with chromic gut sutures. The tissue layers were approximated in the usual manner.

Postoperatively, the rats were maintained on the prepared liquid diet for 10 days. This was done in preference to mandibular immobilization and naso-gastric polyethylene tube feeding.

In all, some 34 rats were surgically attempted, but of these, there were 21 fatalities which occurred either during the surgical procedure or were sacrificed because of the development of postoperative infection. The majority of these fatalities resulted during the early days of the project. Since the introduction of pre- and postoperative antibiotics, and perhaps as the result of improving techniques in the anesthetic and surgical procedures, our fatality rate has decreased markedly. Of the remaining 13 rats, four were used as controls, i.e., resection without a subsequent graft, and nine received anorganic bone grafts following resection.

It was our intention to sacrifice the rats at predetermined intervals and to evaluate the graft site with the object of relating the histological changes with the gross radiographic findings. To obtain suitable histological sections with our present equipment, it would be necessary to decalcify the specimen prior to sectioning and thus "wash out" much of the anorganic graft. As a result, only a very few rats have been sacrificed to date, and these, of necessity, have been out long term specimens. In all, seven rats have been sacrificed, four of which had been previously grafted and the remaining three being controls.

At autopsy it was noted that "clinical union" had resulted in the grafted rats and to a lesser degree in the controls. As a result of the resection, the control rats naturally exhibited a deviation of the mandible to the surgical side. This deviation was absent where grafts had been inserted.

The mandibles of the sacrificed rats were disarticulated, following which adequate radiographs were obtained. Fixation in 10% formalin for 24 hours was followed by decalcification in versene for approximately 3 - 5 weeks. A double embedding technique was employed and the sections were stained with haematoxylin and eosin.

Our histological findings are very limited at this time for want of adequate specimens. We have evaluated the available sections to determine: (1) the presence or absence of foreign-body reaction; (2) the degree of reactive bone formation; (3) the degree of revascularization.

We are certainly not in a position to make any definitive statements, but it must be noted that our findings to date are encouraging in that there has been an absence of foreign-body reaction at the graft site, that reactive bone formation has been satisfactory, and that revascularization has been noted as early as 2 - 3 weeks.

It is anticipated that continuation of this project for another year will permit us to more thoroughly evaluate far more specimens and to come forth with more specific findings.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

- Subject: 1. Further studies on the role of the dental plaque in the caries process.
2. Further studies on the effect of substances removed during the refining of carbohydrates on calcium phosphate solubility.

Grantee: I. Kleinberg

Project: D.A. 78

Amount Awarded: \$4,345.00 - 1959-60

Project I. Further studies on the role of the dental plaque in the caried process.

In the study on the effect of different concentrations of glucose on the pH of dental plaque in vivo (see previous application form), it was decided to include a comparison of the maximum plaque pH falls that can be obtained with each of a range of glucose concentrations in a given individual. Several experiments were carried out on each of three such individuals and in each experiment, the effects with one glucose concentration were compared to those for 5% glucose. By taking the pH values observed for 5% glucose as giving a maximum or nearly maximum pH fall (and considering this to be a 100% pH drop) and then by expressing the pH drop obtained with the different glucose concentrations tested as a percentage of that obtained with the 5% glucose, it was possible to compare not only the given glucose concentration to the effects with 5% glucose in each experiment but also to compare the effects between each of the different concentrations tested in the different experiments. That is, the difference between the fasting pH values and the pH after 5% glucose acted as a "yardstick" in each experiment.

Two maxillary anterior interproximal and two maxillary labial gingival plaques were studied in each of the subjects and in each case, the same selected plaques were studied with each glucose concentration tested. The results are given in figure 1 and indicate that (i) with relatively low glucose concentrations the plaque pH fall is directly related to the glucose concentration (ii) at higher levels, a maximum pH fall is reached and (iii) at the highest levels studied, the pH fall is directly related to the glucose concentration. It is important to note however that this inhibition of the pH fall at the higher glucose concentrations is appreciably less than that found in vitro in saliva-glucose mixtures.

One might point out here that these observations would indicate that if a 5% glucose solution is used to bring the pH of a

given plaque to or near to its minimum level, then the effect of a given substance on the metabolism of the acidogenic bacteria in this plaque could be tested by including a given concentration of this substance in a 5% glucose solution and then determining whether the pH minimum is altered. To determine the effectiveness of the substance being tested, at pH levels higher than the pH minimum, then glucose solutions of lower concentration would have to be used. In other words, these observations suggest a direct method for studying the effect of a given substance on the metabolism of the acidogenic bacteria in a given dental plaque at different pH levels in vivo.

This study has now been completed and submitted for publication and support from the Committee has been gratefully acknowledged.

Since the pH curves obtained with saliva for different glucose concentrations when compared to those for plaque in vivo, showed less inhibition of the plaque pH fall at higher glucose concentrations, it was therefore decided to determine whether this difference could be accounted for by the difference in cell concentration in these two systems. This was done by setting up different saliva-glucose mixtures containing different cell and glucose concentrations, incubating at 37°C for given time intervals and then determining and comparing the pH values produced.

It was found that, at all cell concentrations, the pH fall was directly related to the glucose concentration at very low concentrations; at higher substrate levels, the pH fall reached a maximum while at still higher substrate levels the pH fall was related inversely to the glucose concentration. That is, even for different cell concentrations, these curves showed a typical enzyme-substrate relationship which is in agreement with the findings and the theoretical considerations made previously (Kleinberg, 1958, Ph.D. Thesis; Kleinberg, 1960, J. D. Res., submitted for publication and see above). Where these curves chiefly differed however, is in their response at the very high glucose concentrations; for it was found with increasingly higher cell concentrations in the mixtures, the inhibition of the pH fall at the higher substrate levels progressively decreased so that with the highest cell concentrations studied, only a slight inhibition of acid production occurred - just as was found with dental plaque in vivo. This clearly indicated that the difference in the response found with different glucose concentrations in saliva and in plaque could be attributed to their difference in cell concentration.

It might be pointed out here that from the observations made, there seemed to be some indication that higher glucose concentrations have a greater inhibiting effect on bacterial growth than on bacterial acid production. This would suggest that inhibitors of lysis when tested in a saliva-carbohydrate system could give a quantitatively different result than when tested in a system in which the bacteria are present at a higher concentration (e.g. saliva sediment).

Similar studies were carried out in which the glucose was replaced by fructose. The findings made were similar except that the fructose concentration which gave the largest pH fall was lower than that for glucose and the maximum or nearly maximum pH fall did not extend over as wide a range of substrate concentrations.

This portion of project I is now in preparation for publication. A study of the effect of different levels of the other more common sugars alone and in combination is in progress in conjunction with a study of the effect of unrefined and refined carbohydrates on acid production in vitro and in vivo (see previous application form).

A study was undertaken to determine the effect of different concentrations of urea on the metabolism of the oral bacteria and to then use these results as a guide to a study in vivo. In the in vitro investigation, a study of the effect of different concentrations of urea on the pH produced in incubated urea-saliva mixtures and urea-sediment was carried out. In addition, the effect of different concentrations of urea in the presence of different levels of glucose in the former mixtures was also studied.

In the urea-saliva mixtures, it was found that at the low urea concentrations the pH rise was directly related to the urea concentration, with increasingly higher urea levels, a maximum pH rise was obtained while at the highest levels studied, a slight inhibition of the pH rise was observed. In the urea-saliva sediment mixtures, similar results were obtained except that little or no inhibition resulted at the highest levels.

These results are in contrast to those for glucose in that a maximum pH rise was reached with the urea at a lower molar concentration than the concentration of glucose required to give a maximum pH fall. In addition, there was less inhibition of the pH rise with urea both in the saliva sediment at the very high substrate concentrations than there was a pH fall with the corresponding levels of glucose. When different combinations of concentrations of urea and glucose were studied in incubated saliva, it was found that, in general, when the urea concentration was above a level of approximately 1%, for all concentrations of glucose studied (0 - 30%) the pH of the saliva-substrate mixture rose. That is, with this level of urea, glucose could not cause a pH fall. When the urea concentration was approximately 1% or less, except for glucose concentrations below approximately 0.5 - 1% and above approximately 25%, the pH of the mixtures fell.

These observations indicate that the concentration and the type of substrate or substrates present in the saliva-substrate mixtures would be expected to determine the pH of these mixtures, other factors being equal.

The study in vivo with urea is still in progress but the following observations have been made. When urea was applied locally to selected plaques, as with glucose (except that the pH

change is in opposite directions) the adding of urea caused a pH rise while removing the added urea resulted in the pH falling to its starting level. When urea was added continuously over a period, the fasting steady state pH was disturbed to a new more alkaline level being dependent upon the concentration of urea added. The level of urea that will give a maximum pH rise and the question as to whether any inhibition of the pH rise occurs at the higher urea concentrations has yet to be decided. Increasing the length of time, from a relatively short to a relatively long period, that a given concentration of urea is made available to the plaque bacteria resulted in a greater pH rise and for a longer period for the latter: thus giving a larger area under the pH curve. The results thus far indicate that as with glucose, the concentration and the length of time that a given concentration of urea is available to the plaque bacterial will be important determining factors in the pH of a given plaque.

It should be pointed out here that the same plaque can give a pH rise in the presence of a nitrogenous substrate such as urea or a pH fall in the presence of carbohydrate. Because the pH in a plaque can range from a value above 9.0 to one below 5.0, this would support the view that the same plaque could cause both the development of a cavity by the dissolution of the acid soluble portions of the tooth or the precipitation of calcium and phosphorus from the saliva to form tartar.

Project II. Further studies on the effect of substances removed during the refining of carbohydrates on calcium phosphate solubility.

Under this project, the effect of pH on the solubility of Ca and P in saliva is being studied. At the same time, an attempt is being made to determine how the solubility of the organic material in saliva is influenced by the pH change. The pH range selected was that which would include the pH levels that can be found in dental plaque; i.e. approximately 9.5 - 4.0. Different pH levels in saliva were obtained (a) by allowing bacterial metabolism to proceed for given time intervals and (b) by the adjustment of the saliva pH with acid or base.

The results thus far indicate that the inorganic phosphate concentration changes very little over this wide pH range although there is an indication of a small decrease above pH 7.0. Calcium on the other hand, shows its highest levels at the lower pH range (approximately 4.0 - 5.5) and then decreases slightly between approximately 5.5 - 8.0 and then decreases very sharply above 8.0, clearly demonstrating the decreased solubility of Ca in saliva at higher pH levels. Whether the solubility of Ca is related to its combination with proteins or other organic material in saliva is still undecided at the present time.

It is planned to test the hypothesis that a given saliva has two "critical" pHs - an acid one which would be the pH at which

Ca and phosphate ions are dissolved from enamel, and an alkaline one, the pH at which Ca and phosphate are precipitated onto tooth surfaces as tartar. It is then planned to determine whether organic phosphates and whether unrefined carbohydrate can influence the levels of the critical pHs, especially the acid one, in the hope that eventually a practical method for caries prevention can be found.

The work thus far in Projects I and II has provided further support for the Combined Chemico-Parasitic Theory (see previous application form).

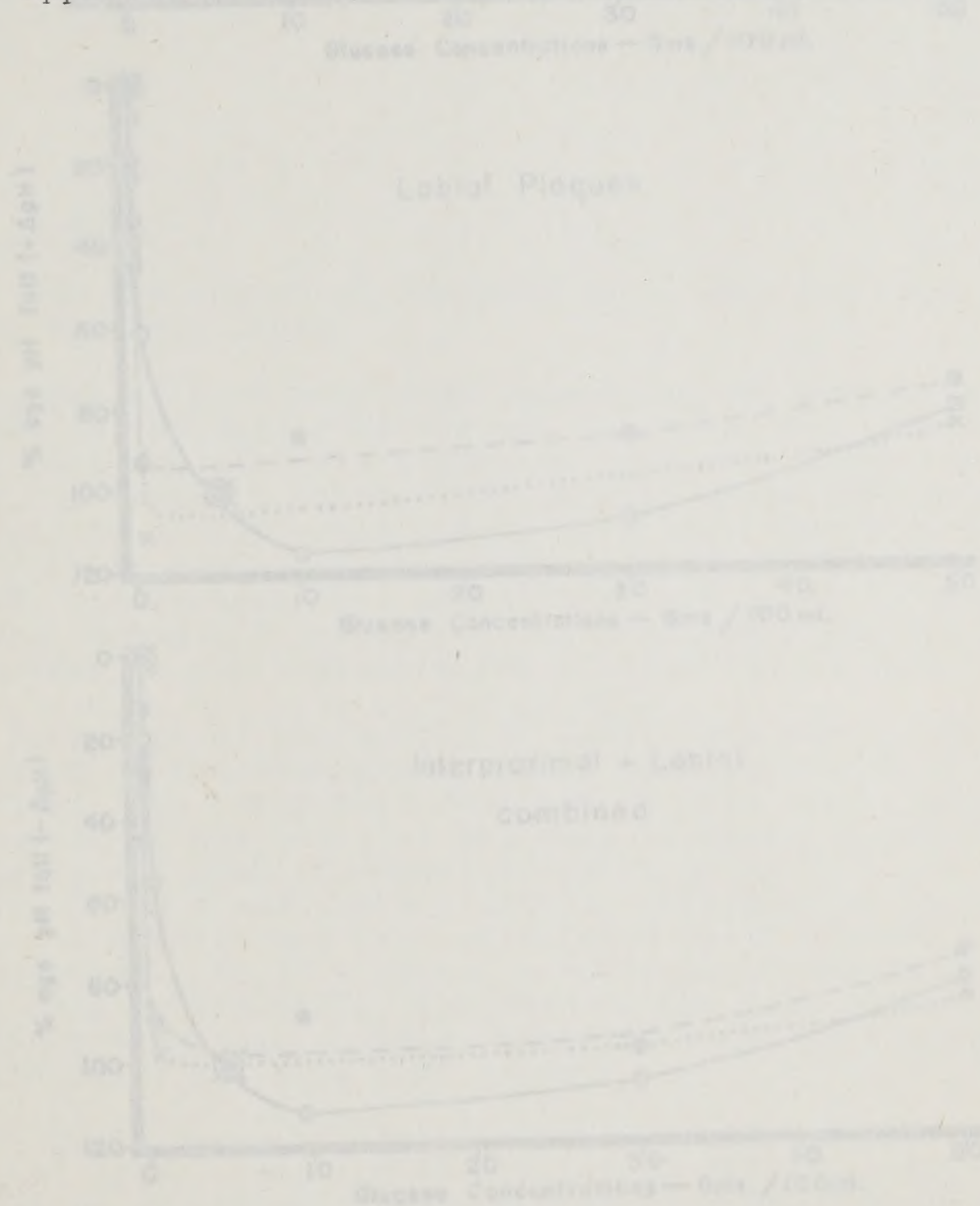


Figure 11 The relationship between the glucose concentration and the percentage increase in interproximal and labial plaque, at levels in each of three subjects. (S.A. — — — — —, S.H. — — — — —, and S.L. — — — — —)

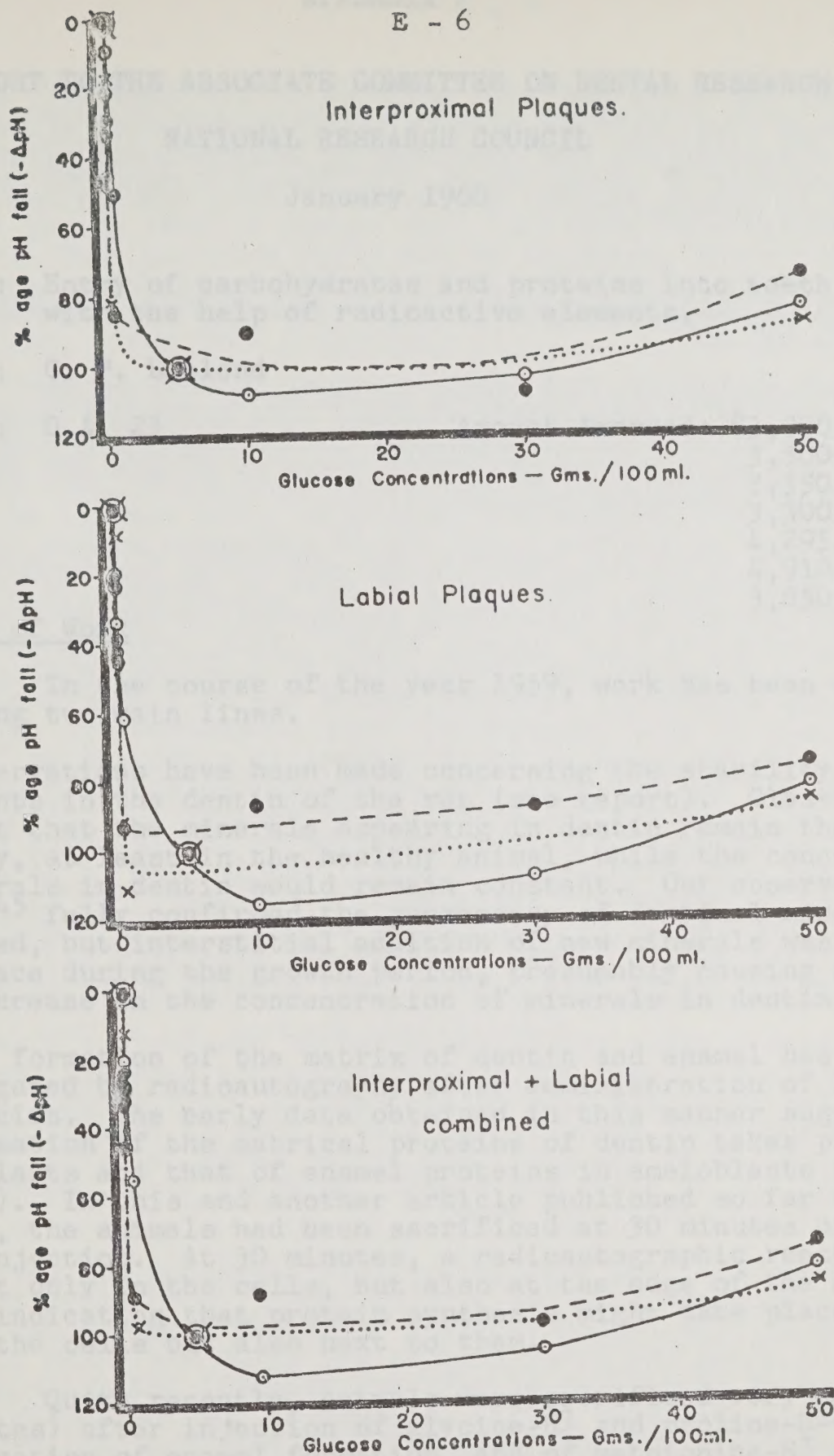


Figure 1: The relationship between the glucose concentration and the percentage decrease in interproximal and labial plaque pH levels in each of three subjects (V.S. — — • — —, E. M.x....., and G.L. — — o — —)

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Subject: Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements.

Grantee: C. P. Leblond

Project: D.A. 23

Amount Awarded:	\$3,000.00	- 1953-54
	3,500.00	- 1954-55
	3,350.00	- 1955-56
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	4,295.00	- 1957-58
	4,910.00	- 1958-59
	3,850.00	- 1959-60

Summary of Work

In the course of the year 1959, work has been carried out along two main lines.

1. Observations have been made concerning the stability of mineral components in the dentin of the rat (see report). Classically, it was felt that the minerals appearing in dentin remain there permanently, at least in the healthy animal, while the concentration of minerals in dentin would remain constant. Our observations with calcium⁴⁵ fully confirmed the permanency of dentinal minerals once deposited, but interstitial addition of new minerals was found to take place during the growth period, presumably causing a progressive increase in the concentration of minerals in dentin.

2. The formation of the matrix of dentin and enamel has been further investigated by radioautography after administration of labelled amino-acids. The early data obtained in this manner suggested that the formation of the matrical proteins of dentin takes place in odontoblasts and that of enamel proteins in ameloblasts (see enclosed reprint). In this and another article published so far on the subject, the animals had been sacrificed at 30 minutes or later after injection. At 30 minutes, a radioautographic reaction was seen not only in the cells, but also at the edge of the matrix - a result indicating that protein synthesis might take place not only within the cells but also next to them.

Quite recently, animals were sacrificed very early (5, 10, 30 minutes) after injection of glycine-H³ and proline-C¹⁴ for the investigation of enamel formation, and of methionine-H³ for the investigation of enamel formation. At 5 and 10 minutes, the results were clearcut: radioautographic reactions were exclusively over the cells. At 30 minutes, however, reactions were again seen at the edge of the matrix, indicating that some of the material

elaborated in the cells had been secreted to the outside. It is concluded from these various results that the proteins of dentinal matrix are formed within the cytoplasm of odontoblasts exclusively, while the proteins of enamel matrix are formed within the cytoplasm of ameloblasts exclusively.

Observations Concerning the Stability of the Mineral Components of Dentin in the Rat

For centuries, the tooth - enamel as well as dentin- has been considered to be completely stable. The tooth was looked upon as a tool well adapted to the grinding of food and, like any common usage tool, was believed to persist as long as it was not worn out by attrition or otherwise damaged.

In the thirties, however, the stability of all organs and tissues was questioned. The classical work of Borsook in North America, Schönheimer and Rittenberg in Germany, revealed that many tissue components are in a dynamic state. Thus, even though in the adult animal, the weight and composition of an organ, such as the liver, remains apparently constant, there may be a continuous loss and replacement of some components. Such components are said to "turn over" and the speed of their renewal is expressed as "turnover rate", (that is, the amount of the component replaced per unit time). Conversely, if stability is, according to Webster's dictionary, "resistance to change", a stable structure is one in which the components do not turn over significantly. The problem to be examined is whether tooth components are stable or turnover. It may be recalled that both enamel and dentin are composed of an organic matrix impregnated with mineral salts. Therefore, stability has to be examined with regard to both matrical and mineral components.

Shortly after the introduction of radioactive elements as a tool in biology, Hevesy and his collaborators observed the passage of radioactive phosphate into mature dentin. They assumed that the mineral components of hard tissues play a role in regulating the mineral metabolism of the body; and, in so doing, mineral ions would pass into and out of bones and teeth. In other words, bone and dentinal minerals would turn over.

Meanwhile, the radioautographic technique was used to follow radiophosphate into the growing tooth. Soon after injection of this element, the radioautographs of dentin showed a reaction predominating along the inner surface and which was taken to indicate the site of deposition of dentinal minerals (Bélanger and Leblond, 1950). Bélanger (1952) followed up the phenomenon at late time intervals and concluded that the reaction band eventually decreases in intensity and perhaps even vanishes, a finding suggesting a turnover of minerals in the labelled dentin and presumably in the rest of dentin as well.

Later, calcium⁴⁵ became available and proved to yield dentinal radioautographs with a higher resolution than, but with the same overall pattern as radiophosphate (Kumamoto and Leblond, 1956). A reaction band appeared on the inner surface of dentin and did not vanish with time. However, a certain degree of reactivity was seen in the rest of dentin too. Was this fact indicative of turnover? This question could not be answered, since the observations were qualitative. Hence, it was decided to repeat the work using quantitative radioautograph, as done by counting silver grains over dentin, after Ca⁴⁵ injection.

Meanwhile, a series of investigations dealing with the stability of the matrix of dentin were underway in this laboratory (Greulich and Leblond, 1954). After injection of bicarbonate-C¹⁴ to 3-day old rats, which were sacrificed at intervals varying from 3 hours to 15 days after injection, the radiocarbon was found to enter the matrix appearing at the inner surface of dentin and, as more and more new matrix was added, the labelled portion was retained without change throughout the experimental period. Thus, the number of grains counted in the emulsion over the radioactive area showed no significant change with time, so that, during the 15-day experimental period, the C¹⁴ labelled component of the matrix had remained unchanged.

Identical observations were made after injection of glucose-C¹⁴ to 3-day old rats (Kumamoto and Leblond, 1958). Even though the experiment was carried out for long periods and the animals were sacrificed as late as six months after injection, the radioautographic reactions showed no change whatever with time. The occlusal surface of the teeth was worn away by attrition, due to the grinding of food against the opposite teeth, but the retained parts showed a radioautographic reaction identical to that observed at early time intervals. Hence, the C¹⁴ component of dentin remained unchanged for at least six months and perhaps indefinitely.

Similarly, a stable reaction was observed in the dentinal matrix of growing incisor teeth in mice after injection of either one of the three amino-acids, leucine-, methionine- and glycine-H³, a fact indicating that the permanent component(s) of dentin is a protein (Carneiro and Leblond, 1959). Since collagen makes up about 95% of the protein material in dentinal matrix and radioautographic reactions were much more intense with glycine, which is abundant in collagen, than with leucine or methionine, which are not, it was concluded that the persisting component of the dentinal matrix is collagen (Carneiro and Leblond, 1959). Unpublished results fully confirmed this conclusion since the same stable pattern was observed after administration of a fairly specific precursor of collagen, labelled proline. It is concluded that at least, as far as its collagen component is concerned, the dentinal matrix is a permanent structure. This conclusion is in line with classical concepts.

In the hope of clarifying the situation with regard to the mineral components of dentin, presumably by means of quantitative

radioautography the following technique was adopted. Calcium⁴⁵ was administered to 3-day old rats, which were sacrificed at various time intervals thereafter. Since stability may be considered to be the absence of turnover, it should exclude entry, as well as loss of minerals in dentin, except of course on its inner surface where more and more dentin is being added. It was therefore, decided to examine the labelled portion of dentin, that is, the deepest layer of dentin immediately after calcium⁴⁵ injection and to determine by means of grain counts whether or not its radioactivity remains constant. If so, dentinal minerals would be stable. Attention was focused on the first molar of rats given a single injection of radioactive calcium when three days old, and sacrificed at various intervals between one day and six months after injection of calcium⁴⁵. The teeth were cut in serial sections and a definite area of the first molar was selected for quantitative investigation.

THE RAT MOLARS

Since the available descriptions of rat molars (Schour and Massler, 1949, p. 119; Mummery, 1933, p. 560) were not sufficiently detailed to make this work possible, it was first found necessary to reexamine the morphology of these teeth. A brief summary of the results follows.

The upper and lower molar teeth were dissected. Photographs of their occlusal surface (Figs. 1-3) clearly showed that each tooth was composed of transvers ridges. Thus, in the first molars (Fig. 1, I), three ridges are arranged in planes perpendicular to the body axis which are oriented forwards and up in lower molars (as shown in Figs. 4 and 13), and backwards and down in the case of the upper molars (Fig. 19). Both have their occlusal plane tilted in a medio-ventral direction.

The upper first molar has three large ridges (Fig. 1), whereas the lower first molar has in addition a small fourth ridge wedged next to the second molar (Figs. 1 and 4). The occlusal surface of each ridge is provided with an enamel-free area, where naked dentin may be seen (as dark material in Figs. 2 and 3). A histological section through such an area shows the enamel-free dentin, as for instance between the two oblique arrows at the summit of the second ridge in Fig. 4. Incidentally, the picture demonstrates that enamel-free dentin is present before the tooth erupts. This is also shown in Figs. 2 and 3 which were obtained from a 12-day old animal, that is, at a time when eruption has not taken place but is imminent (in most of the animals of the colony used).

The second and third molars are also composed of ridges (Fig. 1), which remain somewhat smaller than the first molar, as may be seen for the lower (e.g. Fig. 15) and upper molars (e.g. Fig. 25).

Soon after the molars erupt (Figs. 9 and 19), their surface wears out by attrition due to the grinding of food between the upper and lower molars. This may be seen by comparing the height of the ridges in Figs. 19, 21 and 25. It seems that the attrition proceeds slightly faster for the upper than for the lower molars.

MATERIALS AND METHODS

In the experiment to be reported, 3-day old rats received a single subcutaneous injection of calcium⁴⁵. Some of the animals sacrificed up to nine days after injection had their teeth fixed and sectioned using routine embedding technique and coated radioautographs were used; others sacrificed 1 - 6 months after injection had their teeth embedded in plastic and contact radioautographs were used to trace the radiocalcium.

Coated Radioautographs

Three litters of 3-day old RCH rats (weighing 6 - 6.5 gm. in litter 1, 7 - 9.5 gm. in litter 2 and 7 - 8.5 gm. in litter 3) were each injected subcutaneously with a dose of $0.1 \mu\text{c Ca}^{45}\text{Cl}_2$ in 0.036 cc solution per gram body weight. They were then returned to their respective mothers. The animals were weighed at the time of injection and one or two from each cage were sacrificed, 1, 3, 6 or 9 days later. Jaws and other tissues were removed and fixed in alcohol-formal (10 volumes of the 40% commercial formalin and 30 volumes of 95% alcohol) for 24 hours and then stored in the same solution diluted by half with 95% alcohol. Upper and lower jaws from the left side were trimmed (the incisors being removed) and embedded in paraffin. The blocks were sectioned semiserially at 6μ taking one section out of every 75. The unstained, semiserial sections were given two coats of 1% celloidin. In addition, 3 - 6 more test slides were prepared for finding out the optimal exposure. The test slides were radioautographed according to the "coating technique" by dipping into Kodak NTB 3 melted emulsion (Messier and Leblond, 1958) and developed at various time intervals thereafter.

In previous work with rats given a single dose of Ca^{45} when three days old, the exposure was prolonged until a distinct radioactive band appeared over dentin (Kumamoto and Leblond, 1956). However, for grain counting, it was found necessary to reduce the intensity of the reaction by shortening the exposure. Besides facilitating counting, this procedure improves accuracy, since the lower the exposure, the better the resolution (Gross *et al.*, 1951). The tests reveal that a two-day exposure yielded radioautographs with an intensity of reaction adequate for grain counting. The semiserial sections were then coated with emulsion as above and developed two days later.

After examining the unstained radioautographs of rat molar teeth, it was decided that grains would be counted only over

the enamel-free dentin at the occlusal surface of the second ridge of the first molar. Counts were carried out over the whole thickness of the dentin, that is, starting from the outer surface of dentin up to predentin (which itself does not elicit a significant reaction in the emulsion, Kumamoto and Leblond, 1956).

Counting the silver grains was carried out by means of an ocular grid, consisting of a large square divided into 400 small squares (20 x 20). At the magnification used (oil immersion, x 800), the sides of the small squares measured 5.8μ .

The grid was placed over the area of enamel-free dentin selected, in such a way that one of the lines of the grid followed the outer edge of the dentin. This area was then drawn to scale using graph paper, and the squares of the grid were drawn in, as done in Zone I at the right of Fig. 5 (Z1). It is apparent in this case that a series of rows composed of two squares each extend parallel to the outer edge of dentin as far as predentin. Counts of grains were then made and recorded for every row, the rows being numbered 1, 2, ... starting from the outer edge of dentin.

To standardize the counting, it was decided that, of the silver grains appearing on the lines demarcating a square, only those on the left and lower borders would be counted. Furthermore, when aggregates of grains were found, an attempt was made to determine from the outline how many grains were present. If the aggregate was oval or spherical a count of one was given. If the outline suggested that two or more grains were present, a corresponding count was made, three being usually the maximum number recorded. In case of doubt, the number expressed was prejudiced toward a lower rather than a higher count.

Once a series of squares had been counted, as in Zone I (Fig. 5), the orientation of the grid was changed to bring its lines parallel to another portion of the outer surface of dentin, as done for Zones II and III. To avoid duplication of counts, small wedge-shaped areas (dark in Fig. 5), were left uncounted when the orientation of the ocular grid was changed. The fact that the dentin-predentin junction sometimes did not coincide with the border of one of the rows, as in Zones II and III, was taken into consideration in determining average grain concentrations, and only areas of actual dentin were used for calculation (Fig. 5).

The results for all the first rows were averaged, then those for the second row and so on. The data were recorded as mean grain number per square for each row in each animal.

The background count was recorded in eight slides (400 squares each) and found to average 0.13 grain per square. This was considered a low enough figure to be neglected.

Contact Radioautographs

Three-day old rats were injected subcutaneously with a dose of $1 \mu\text{C Ca}^{45}\text{Cl}_2$ in 0.02 solution per gram body weight as above and sacrificed in groups at 1, 2, 3, 4, 5, $5\frac{1}{2}$ and 6 months after injection. The skull was fixed in alcohol-formal for 24 - 48 hours; the jaws were disarticulated, trimmed of connective tissue and cranial and orbital bones. They were then thoroughly dried in a 37°C oven for a few days. Subsequently, they were placed in Bioplastic monomer for a minimum of 24 hours. Next each jaw was transferred into a separate glass vial. Bioplastic, to which catalyst had been added (6 drops catalyst to a paper cupful of Bioplastic) while stirring so as to avoid bubbles, was poured into the vials. The vials were kept at room temperature for a few hours to gel the Bioplastic, transferred to a 37°C oven and kept there 3 - 5 days until the Bioplastic hardened. The glass vials were allowed to cool at room temperature and were then shattered.

The plane of section of each plastic-embedded jaw was determined and the plastic abraded on a motor-driven sanding belt, care being taken to cool it with water or spraying with ethyl chloride. If the section was small, it was stuck on a glass slide with Pyseal wax to aid manipulation. Thus the plastic section could be ground down to a thickness of 2 - 3 mm.

Thereafter, the surface of the water-moistened section was abraded by hand, between two carborundum grindstones (Nos. 196 and 107). Periodically, the surface of the grindstones was cleaned and abraded using 60 mesh carborundum powder. The thickness of the sections was of the order of 100 - 200 μ .

The sections were exposed to photographic film (Ortho) in an x-ray cassette as indicated previously (Gross et al., 1951).

For photographing, the specimens were placed in a bath of immersion oil so that surface scratches did not show.

RESULTS

Grain Counts in Coated Radioautographs of Dentin in Rats Sacrificed 1 - 9 Days After Ca^{45} Injection

During the experiment, the animals gained weight at a normal rate, though somewhat more slowly in litter 1 than in litters 2 and 3, and the thickness of dentin increased with time (Table 1). The grain counts performed over the enamel-free dentin in the second ridge of the first molars are presented in Fig. 6, as mean number of grains per $34 \mu^2$ squares ($5.8 \times 5.8 \mu$) at various distances from the outer surface of dentin.

At one day after injection (Fig. 6) radioactivity was present throughout the thickness of dentin in all animals. The maximum concentration of radioactivity (shown in Fig. 6 by a small black triangle for each animal) is fairly close to the outer surface of dentin, whereas the amount tends to decrease away from it, that is, a predentin and the pulp are approached. (The right hand side extremity of the graph for each animal includes a letter used to refer to this animal and, to the left of the letter a short vertical bar indicating the position of the junction between dentin and predentin.)

At three days after injection, radioactivity is again present throughout dentin (Fig. 6), although the thickness of dentin has increased in the interval (Table 1). At six and nine days, the trend continues, that is, radioactivity extends throughout dentin (Fig. 6). Adding up the number of grains counted in all the rows from the outer edge to predentin, yields the total number of grains throughout the thickness of dentin (Table 2). The total number of grains increases with time, a fact indicating a continuous increase in the radiocalcium content of dentin (Table 2). The radioactivity present in the dentinal tissue deposited towards the end of the experiment (at the right of each graph), is, however, lower than that found in the dentinal tissue deposited towards injection time. With time, the peaks remain located at about the same distance from the outer surface of dentin, whether the animals are sacrificed soon or late after injection. Counts over the upper molar suggest a progressive increase in the height of the peaks, thus indicating the addition of more and more radioactivity with time. However, this phenomenon is not clear in lower molars.

Densitometry In Contact Radioautographs of Dentin In Rats Sacrificed 14 Days - 6 Months after Ca⁴⁵ Injection

Examination of the photographs of the polished jaws and corresponding radioautographs of lower (Fig. 9 - 18) and upper molars (Fig. 19 - 28), as well as densitometric measurements at the base of the ridges (Table 3) show no significant change in the intensity of the reaction with time.

The photographs reveal that the summits of the ridges which are radioactive at 14 days (Figs. 10 and 20) are soon lost (see Figs. 16 and 26, for instance), a phenomenon due to attrition of the occlusal surface.

Incidentally, portions of teeth that develop long after the time of injection may be radioactive. This was the case with the roots of the first molars or with the third molars (Fig. 22).

DISCUSSION

It has been pointed out above that stability may be defined as the condition of a tissue component with no turnover rate. The present investigation consisted in examining whether or not dentin exhibits such stability. If so, neither entry nor exit of minerals would take place in this layer once it has been laid down, so that its radioactivity would remain constant at various time intervals after injection. As for the dentin formed during the days preceding the injection of calcium⁴⁵, it should also be stable and, therefore, would not be expected to take up radioactivity.

Immediately after Ca⁴⁵ injection, this element is deposited in dentin, with predominance on the inner surface. If stable, the labelled material deposited should retain the same radioactivity with time. The concentration of calcium⁴⁵ was therefore measured at successive time intervals after Ca⁴⁵ injection, first by grain counts and later by densitometric measurements of radioautographic reactions. Grain counts (Fig. 6) revealed that the radioactivity peak does not decrease with time and, in fact, the trend is towards an increase. At later intervals (1 - 6 months) the amount of radioactive material shows no decrease either, as indicated by examination of photographs (Figs. 9 - 28) or by densitometric measurement of the intensity of the reaction (Table 3). The only exception is the fact that wear and tear at the occlusal surface removes some dentin as well as the included radioactive material. However, in the regions which are not subjected to wear and tear, the radioactive material persists without loss for at least six months and perhaps indefinitely. Hence, the minerals of the labelled layer are being retained permanently. Assuming that the minerals in the rest of dentin behave in the same manner, it is concluded that once minerals are taken up in dentin and the animal is under adequate conditions of housing and feeding, as was the case in our experiments, these minerals remain in situ permanently.

The other facet of stability as defined above is that no new material should be added to the dentinal minerals once they have been deposited. However, whereas the animals show a peak of radioactivity (Fig. 6) presumably corresponding to the dentin formed incrementally immediately after injection, labelled material is also deposited in the dentin formed earlier since radioactivity is seen on the graphs between the peak and the outer surface of dentin. This result confirms the finding of a gradient of reaction between the Ca⁴⁵ reaction band and the outer surface of dentin (see Fig. 1 in Kumamoto and Leblond, 1956).

The appearance of radioactive material in preformed dentin may be due to either an addition of further minerals to this tissue or merely to an exchange of the circulating radioactive calcium ions with the pre-existing calcium ions at the surface of the crystals of dentin. However, a glance at the 1-day diagrams (Fig. 6) indicates that the amount of radioactivity entering the outermost dentin was

equal to about half or even two-thirds of that present at the peak. Since newly deposited crystals are labelled throughout, while exchange labelling would affect the crystal surface, the high radioactivity of the outermost dentin appears too high to be explained by an exchange process. There must also be addition of further minerals, that is, interstitial deposition. The same phenomenon would account for a trend towards the height of the peak increasing with time, as shown particularly for the upper molars (Fig. 6). It is therefore felt that more and more calcium is deposited with time in the preformed dentin of very young rats.

This conclusion is in line with gross observations showing that the dentin of 3-day old rats is relatively fragile and, also, can be easily sectioned with the paraffin microtome. This remains true up to the age of 10 - 12 days, although by that time the hardness had increased and sectioning had become more difficult - both facts suggesting a greater concentration of minerals. In animals aged 12 - 17 days, it is still possible to section dentin if the teeth are embedded in celloidin. Sectioning is no longer possible by these techniques in older animals. With the 1 - 6 month old rats used in the second experiment, the teeth had to be embedded in plastic and polished mechanically in order to prepare the contact radioautographs presented in Figs. 9 - 28. The conclusion is that mineralization of dentin, that is, the concentration of hydroxyapatite increases with age. This increase is satisfactorily explained by interstitial deposition in preformed dentin. In animals aged one month or more, gross examinations showed no significant differences in the hardness of the teeth with age, so that probably the influx of minerals becomes very small or nil.

Examination of microradiographs of dentin reveals an unexpected complication. If one knew that minerals are first deposited incrementally and, later, more is added interstitially, he would expect that density of minerals is less in the area of incremental deposition, that is, at the inner surface of dentin. Surprisingly enough microradiographs prepared in Dr. Lacroix' laboratory reveal that minerals are fairly evenly distributed throughout dentin. This observation requires that 1) during incremental deposition, the dentin formed at a given time acquires minerals in a concentration equal to that reached by the previously formed dentin; 2) interstitial deposition occurs throughout dentin, thus raising the mineral concentration equally in preformed and recently formed dentin.

Such a conclusion requires a systemic control, such as, for instance, the blood level of calcium or other factors inducing the precipitation of an increased amount of minerals as age increases.

SUMMARY

Recent studies of dynamism versus stability of body constituents has invited reexamination of calcium metabolism. The present investigation deals with Ca^{45} turnover in teeth of rats injected at three days of age. Specifically, quantitative work was done on the enamel-free dentin of the second ridge of the first molars.

In one experiment, 20 animals were each given $0.1 \mu\text{c}$ Ca^{45}/gm and sacrificed 1, 3, 6, and 9 days after subcutaneous injection. After fixation in alcohol-formol, the jaws were histologically processed, sectioned semiserially, and radioautographed. Quantitative counts of silver grains, indicating distribution and concentration of radioactivity were made.

In the second, 21 animals were each given $1 \mu\text{c}$ Ca^{45}/gm and sacrificed in threes 1, 2, 3, 4, 5, $5\frac{1}{2}$ and 6 months later. Their jaws were fixed in alcohol-formol, then dried, embedded in Bioplastic and thin sections ground along a plane through the molars and incisors. Contact radioautographs (all correspondingly exposed) were made. Densitometric readings were taken.

Results show that, once incorporated, not only is Ca^{45} stable, insofar as the maximum band of radioactivity remains at the same constant distance from the free edge of the dentin, but also, the amount of radioactivity actually increases up to at least nine days after injection. Densitometric readings show that from one to six months after injection there is no change in the amount of radioactive Ca^{45} present. It appears that a high Ca^{45} level in molars is reached by one month and that this plateau is maintained.

TABLE 1

EXPERIMENTAL PLAN

Time between sacrifice and injection	Litter number	Animal	Weight at autopsy (gm)	Mean thickness of dentin in area counted (μ)	
				Upper molar I	Lower molar I
1 day	1	A	7.5	28	25
	1	B	8	35	32
	2	C	9	41	-
	2	D	10.5	41	38
3 days	1	A	9	58	78
	1	B	10	67	-
	2	C	12.5	64	61
	2	D	12	-	71
6 days	1	A	13	116	-
	1	B	12.5	162	55
	2	C	18	203	116
	2	D	19	128	163
9 days	1	A	20	209	-
	1	B	20.5	-	191
	3	G	21.5	197	-

TABLE 2

TOTAL NUMBER OF GRAINS ACROSS DENTIN

(expressed per 5.8 μ wide column)

Time between sacrifice and injection	Litter number	Animal	Total number of grains across dentin (per 5.8 μ wide column)	
			Upper molar I	Lower molar I
1 day	1	A	15.5	14.1
	1	B	13.5	25.8
	2	C	20.1	-
	2	D	25.5	18.4
	Mean		18.7	19.4
3 days	1	A	17.8	40.3
	1	B	30.0	-
	2	C	31.1	28.7
	2	D	-	56.1
	Mean		26.3	41.7
6 days	1	A	61.0	-
	1	B	42.9	12.3
	2	C	143.6	69.8
	2	D	107.9	100.5
	Mean		88.8	57.6
9 days	1	A	143.3	-
	1	B	92.8	120.7
	3	G	153.5	-
	Mean		129.9	

TABLE 3

DISTANCE OF THE PEAKS OF RADIOACTIVITY

FROM THE OUTER SURFACE OF DENTIN

Time after Ca ⁴⁵ injection (days)	Mean thickness of dentin (μ)		Mean distance of maximal grain concentration from outer surface of dentin (μ)	
			<u>Mean</u>	<u>Extremes</u>
1 Upper	34		17	12-40
Lower	32		17	12-40
3 Upper	56		23	12-23
Lower	73		23	17-29
6 Upper	122		35	17-35
Lower	128		35	23-52
9 Upper	203		23	12-23
Lower	191		18	18

TABLE 4

MEAN DENSITY READINGS OF THE DENTINAL RADIOACTIVITY
AT THE BASES OF THE SECOND RIDGE OF THE FIRST MOLARS
IN CONTACT RADIOAUTOGRAPHS
(relative densities)

Age (in months)	Upper molars	Lower molars
1	67	78
2	70	80
3	60	88
4	71	75
6	45	83

Fig. 1 Dissection of left upper and lower molars from an 18-day old rat.
The first (I), second (II) and third (III) molar of each jaw are composed of ridges which are numbered from front to back with arabic numerals.
In the 18-day old rat the cusps of the first upper and lower molars have just pierced the oral epithelium. Dissection involved stripping away the oral epithelium and connective tissue.

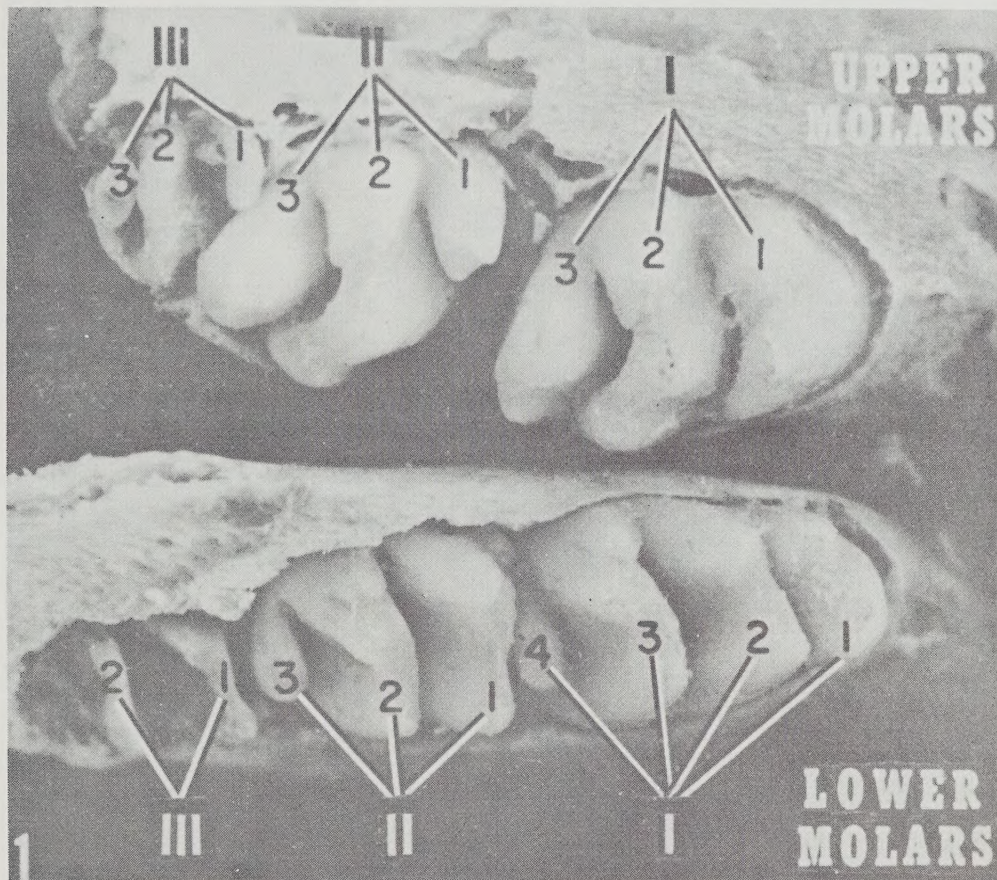
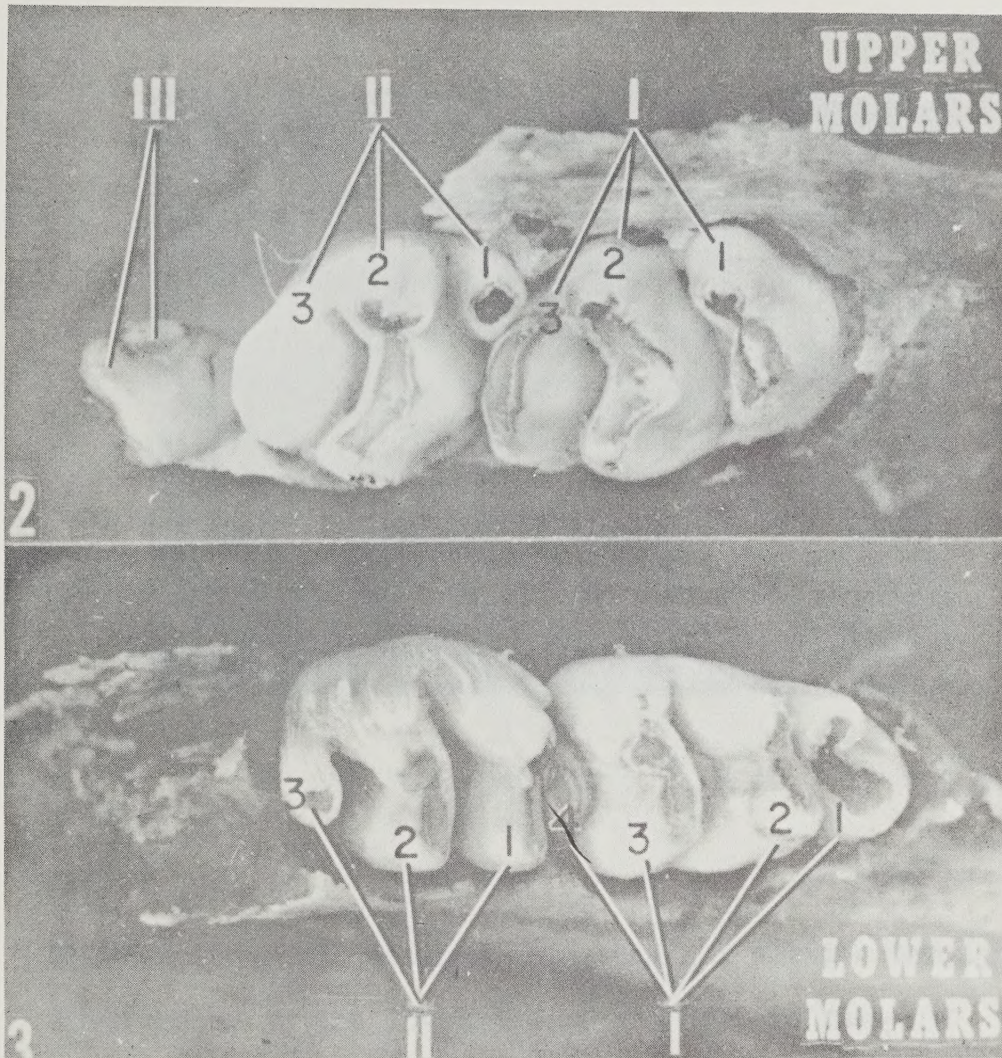


Fig. 1 Dissection of left upper and lower molars from an 18-day old rat.

The first (I), second (II) and third (III) molar of each jaw are composed of ridges which are numbered from front to back with arabic numbers.

In the 18-day old rat the cusps of the first upper and lower molars have just pierced the oral epithelium. Dissection involved stripping away the oral epithelium and connective tissue.



Figs. 2 and 3 Dissection of left upper (Fig. 2) and lower (Fig. 3) molars from a 12-day old rat.

This preparation shows the enamel-free dentin as a dark zone on the occlusal surface of each ridge.

In the 12-day old rat none of the molars has erupted. Dissection involved stripping away the oral epithelium and connective tissue.

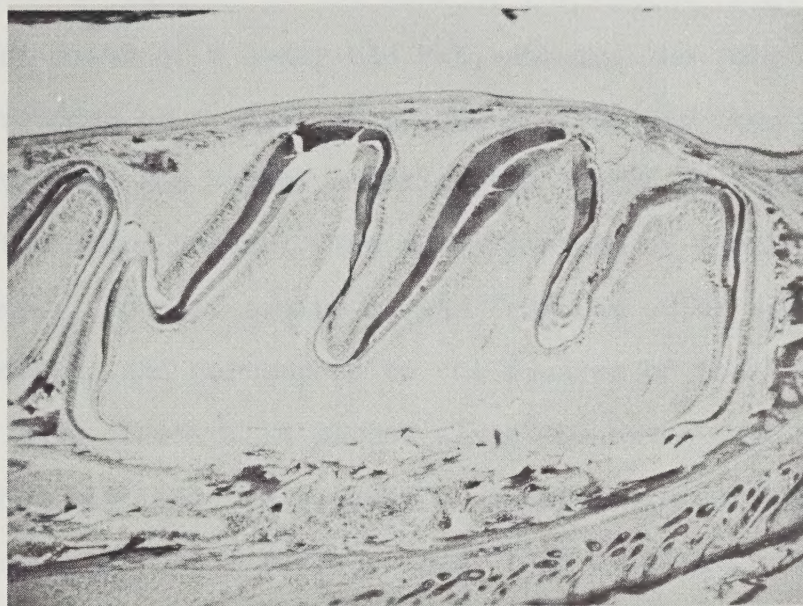
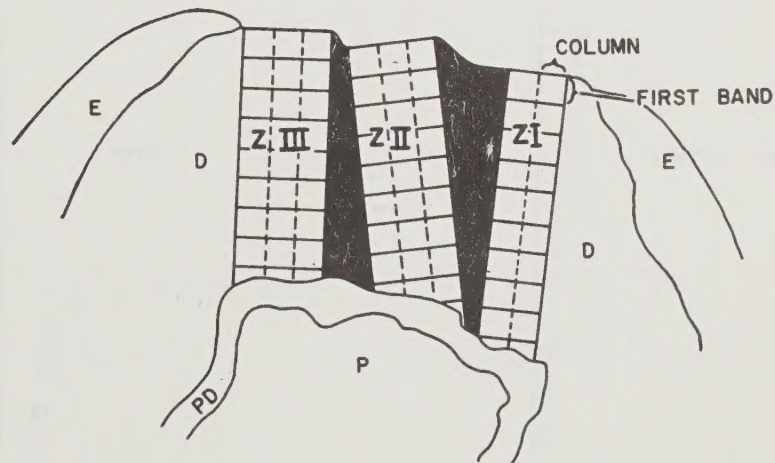


Fig. 4 Hematoxylin-eosin stained, longitudinal section through the first lower molar in the lower jaw of a 6-day old rat.

The oral epithelium is seen at the top (OE) and the skin below the jaw is seen in the lower right corner. The bony trabecules of the developing mandible are scanty at that age but may be distinguished in several places, particularly at right (M). The first molar (I) consists of 4 ridges which are numbered 1 - 4 from front to back. Each ridge is covered by the dark staining enamel (E) with its ameloblasts (A) and within, by the grey staining dentin (D) and its odontoblasts (O). At the apex of the second ridge, it is possible to see an area of dentin which is not covered by enamel (between the tips of the two oblique arrows). Radioautographic grain counts were carried out in such areas.

Fig. 5 Schematic diagram of the apex of the second ridge of the lower first molar of a 6-day old rat, showing the pulp (P, lower center) surrounded by a layer of predentin (PD) and then the fairly thick dentin and the enamel covering the dentin at right and left but not in the upper center. The outer surface of dentin is thus free of enamel. Rows of square extend from the outer surface of dentin to predentin and correspond to the squares of the ocular micrometer, the edge of which is placed along the outer surface of dentin in such a way that the columns of squares are perpendicular to the area of the outer surface under consideration. In zone I (ZI) two columns composed of 9 squares each are drawn. Zones II and III contain three columns each. The rows of squares are numbered from outside pulpward.



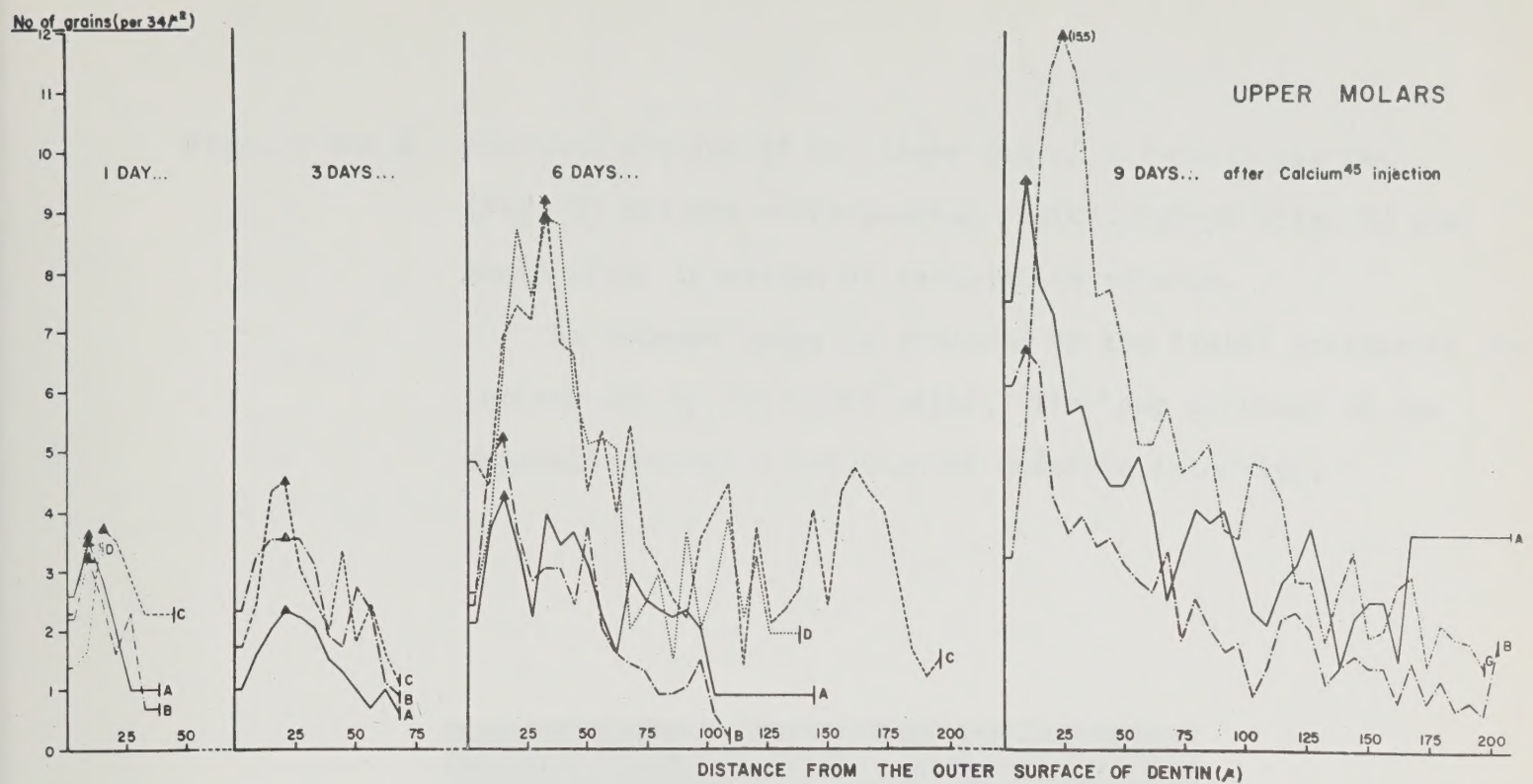
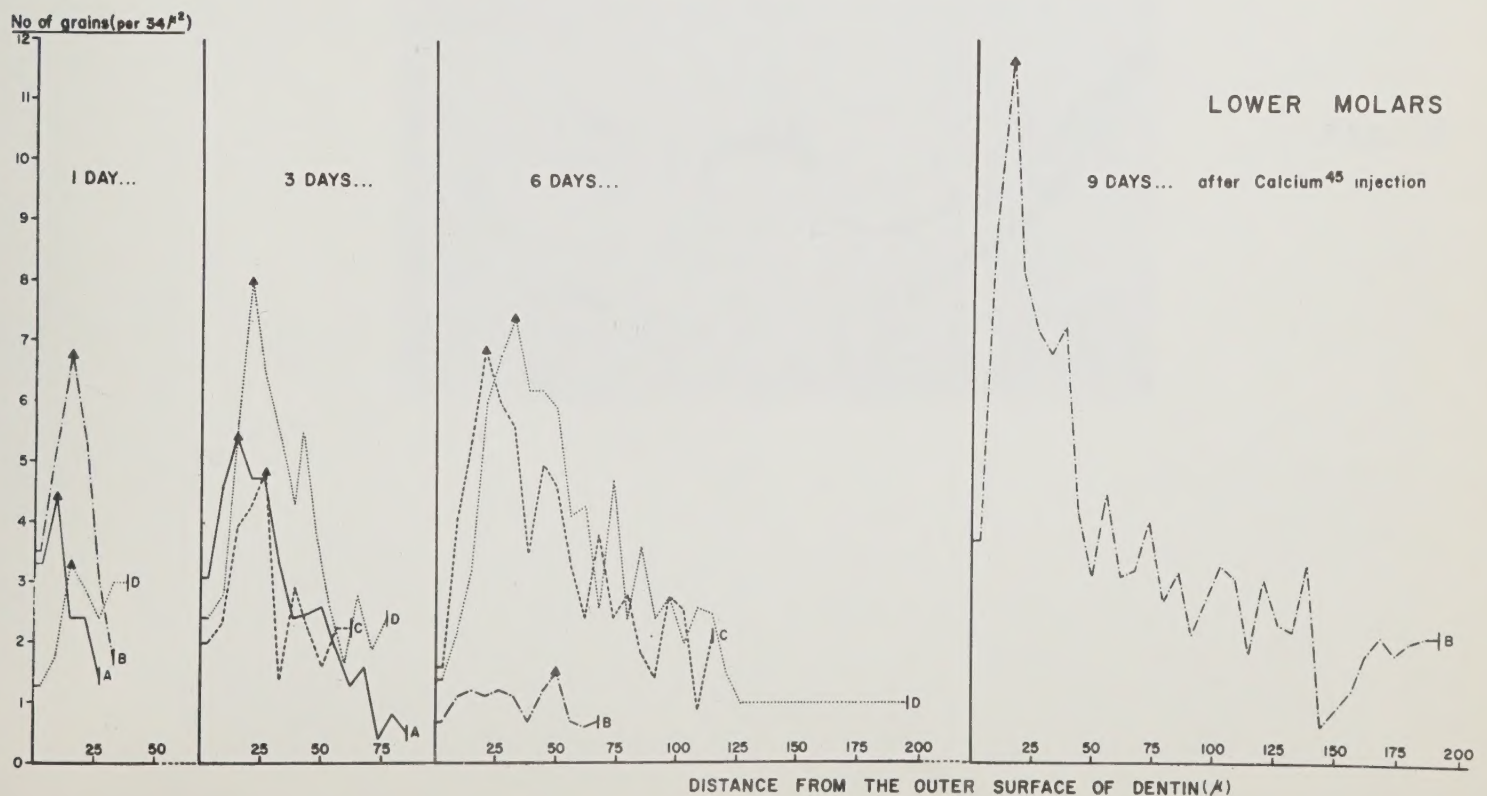


Fig. 6.

Number of grains found in the emulsion over dentin at various distances from the outer surface of dentin at 1, 3, 6 and 9 days after calcium⁴⁵ injection.

The diagrams above represent the data obtained using the upper molars whereas the data below corresponds to those obtained from lower molars.

The highest points on the graph (that is, the greatest cone of radioactivity) is indicated by a small black triangle.



Figs. 7 and 8 Polished section of the lower jaw of a 1-month old rat (Fig. 7) and the corresponding radioautograph (Fig. 8) one month after injection of radioactive calcium.

An intense image is produced by the distal portion of the incisor and by the first molar. The bony portions of the mandible produce a reaction of moderate intensity.

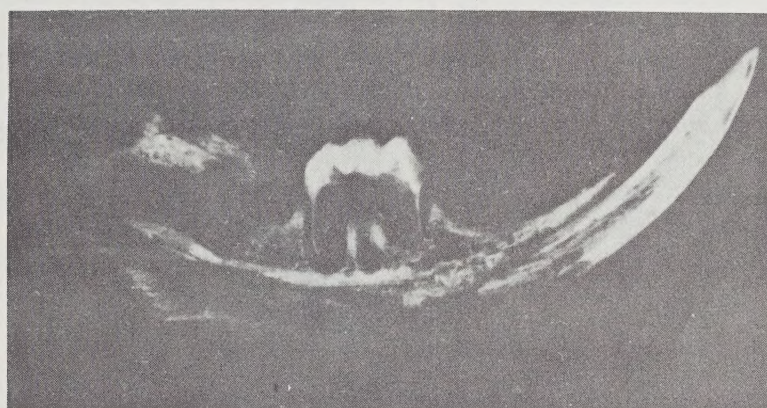


Fig. 7

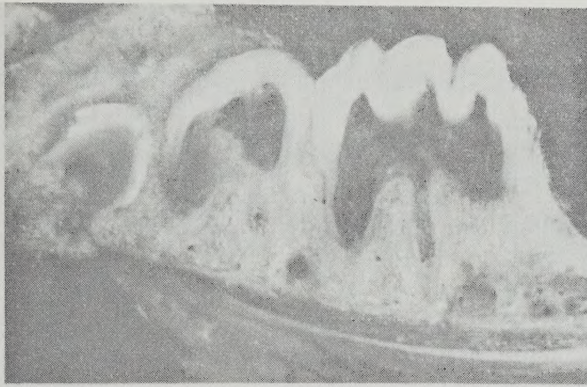


Fig. 8

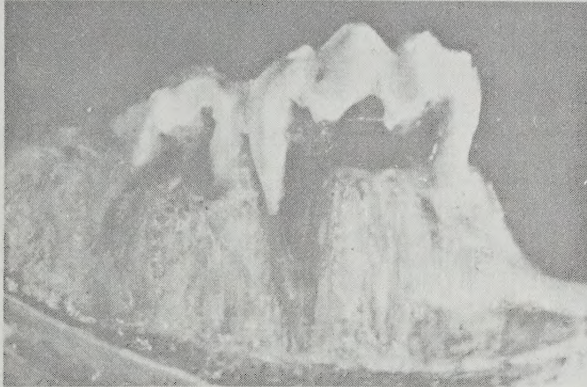
Figs. 9 - 18 Comparison of the sections of lower molars of rats aged 1 - 6 months (Figs. 9 - 17) and the corresponding radioautographs (Figs. 10 - 18).

Figs. 9, 10 - 1 month
Figs. 11, 12 - 2 months
Figs. 13, 14 - 3 months
Figs. 15, 16 - 4 months
Figs. 17, 18 - 6 months

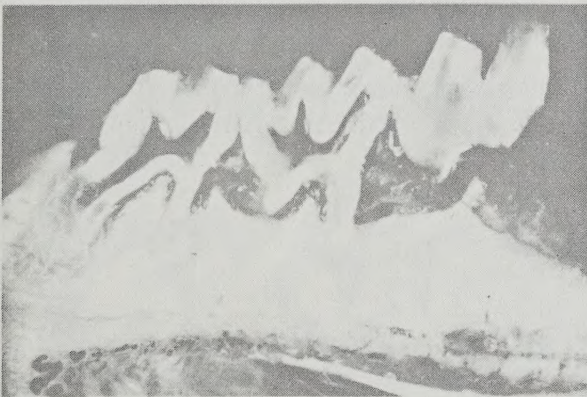
The molars show an intense reaction of the crown region and a slight one of the roots. The portions corresponding to the apex of the ridges and which are intensely radioactive at the early time interval (Fig. 10) are gradually eroded with time so that at 4 and 6 months, the central portions of the ridges are composed of non-radioactive material. In the corresponding photographs (Figs. 15 and 17) it may be seen that these areas are filled with calcified material (that is, secondary dentin). There is no apparent change in the intensity of the reactions of the lateral portions of the ridges with time.



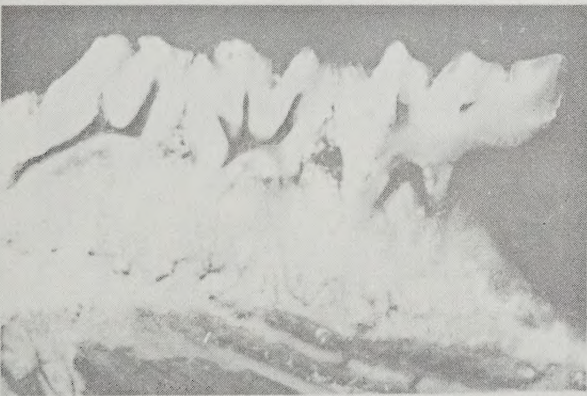
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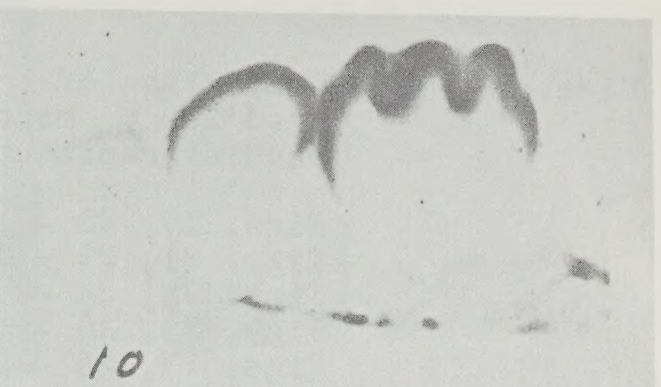
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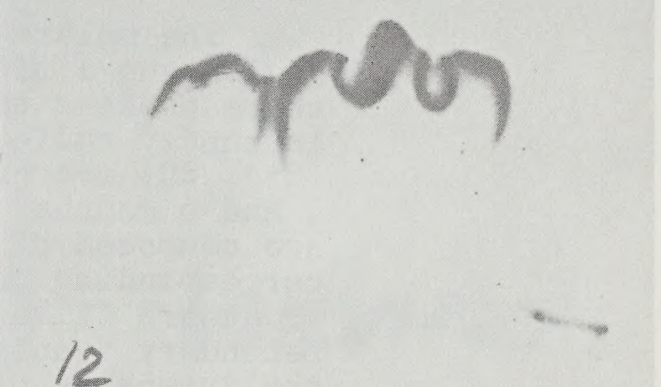
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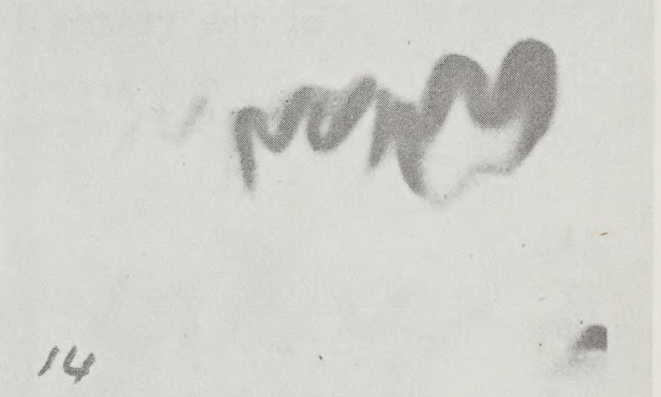
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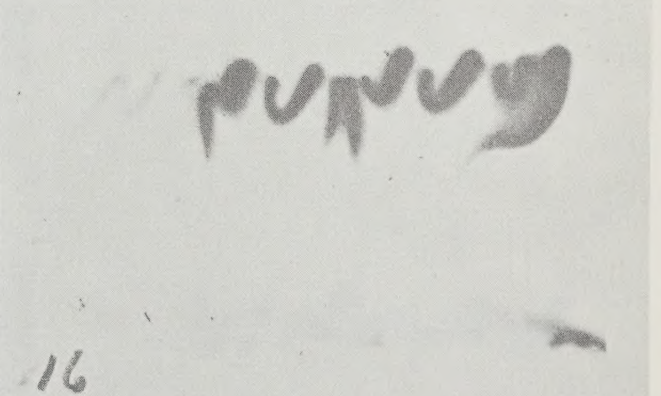
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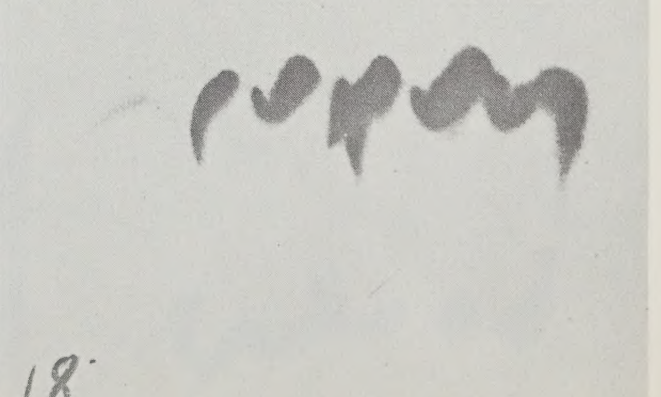
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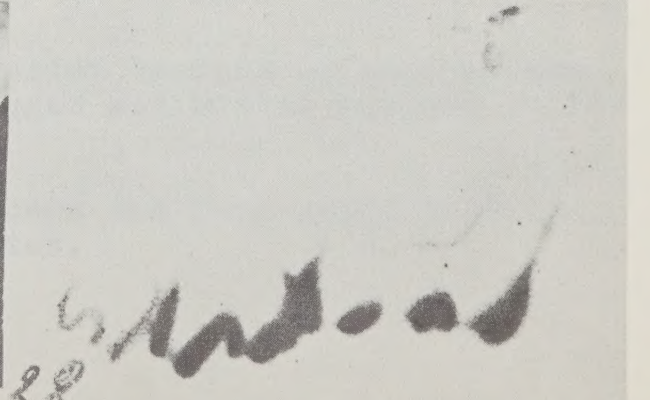
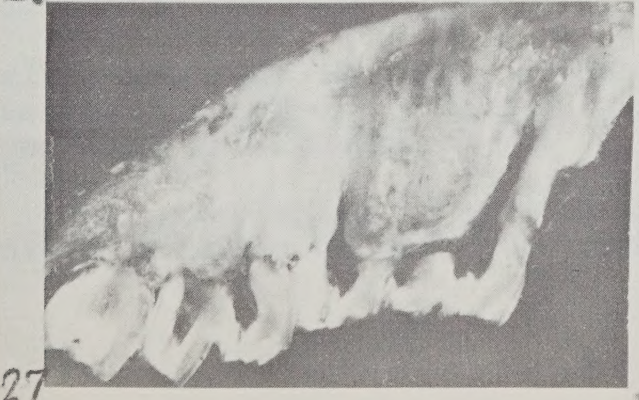
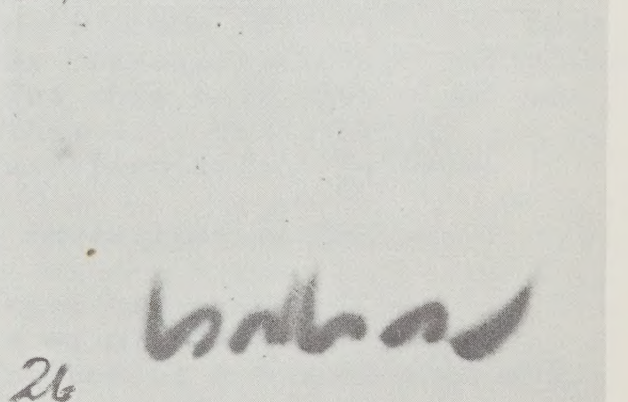
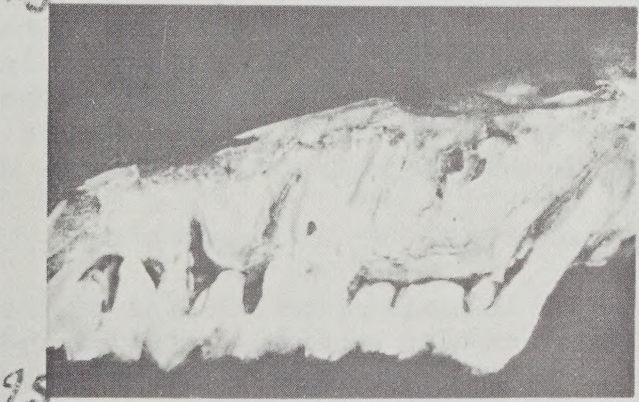
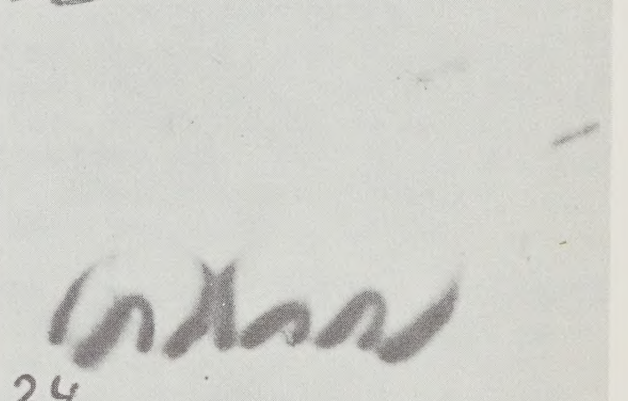
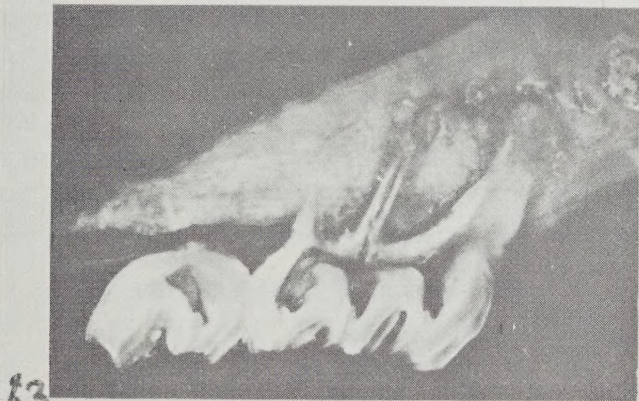
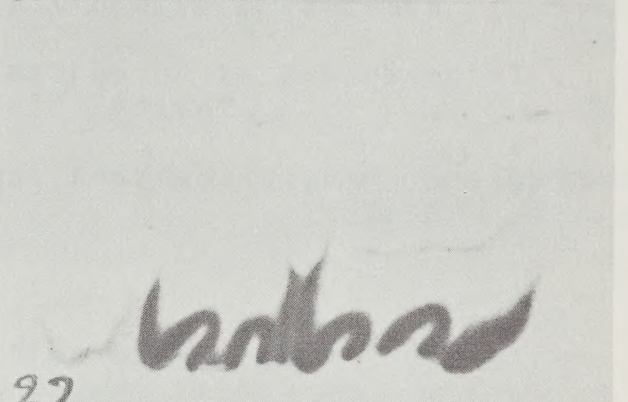
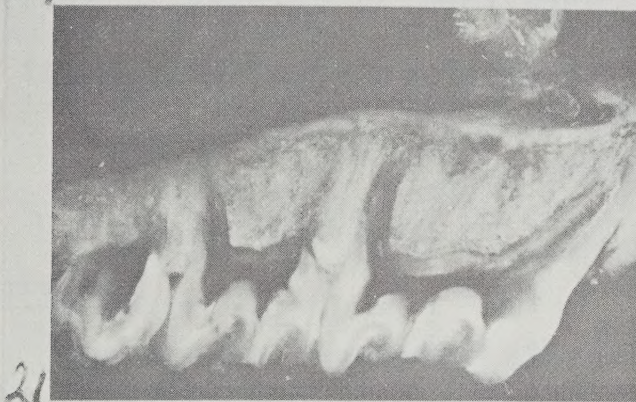
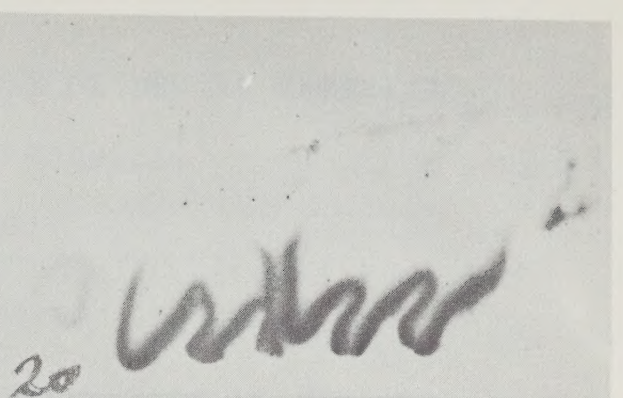
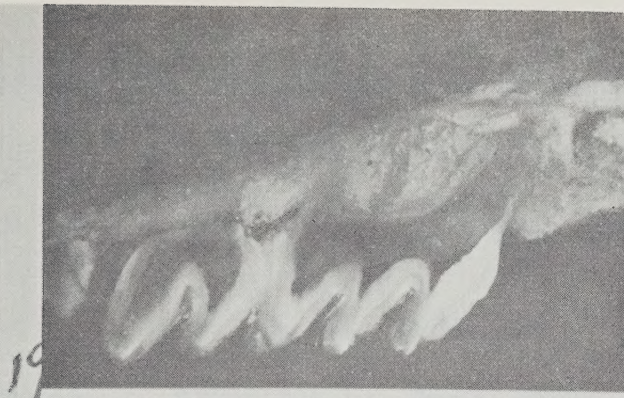
18

Figs. 19 - 28 Comparison of the sections of upper molars of rats aged 1 - 6 months (Figs. 19 - 27) and the corresponding radioautographs (Figs. 20 - 28).

Figs. 19, 20 - 1 month
Figs. 21, 22 - 2 months
Figs. 23, 24 - 3 months
Figs. 25, 26 - 4 months
Figs. 27, 28 - 6 months

The molars show an intense reaction of the crown region and a slight one of the roots. The portions corresponding to the apex of the ridges and which are intensely radioactive at the early time interval (Fig. 20) are gradually eroded with time so that at 4 and 6 months, the central portions of the ridges are composed of non-radioactive material. In the corresponding photographs it may be seen that these areas are filled with calcified material (that is, secondary dentin). There is no apparent change in the intensity of the reactions of the lateral portions of the ridges with time.

The attrition seems to be more pronounced on the occlusal surface of the upper than of the lower molars.



APPENDIX G

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Name: Dr. E. M. Madlener
Institution: Faculty of Dentistry, University of Toronto
Project Title: DA-71, "The Characterization of an Experimental 'Fusospirochetal' Lesion".

Amount Awarded: \$4,640.00 - 1958-59
4,700.00 - 1959-60

Introduction

The specific peripheral position of fusiforms and spirochetes in "fusospirochetal" lesions in humans and in guinea pigs has been used as one of the criteria of the similarity of "fusospirochetal" infections in these two species, (Rosebury and Foley, J. D. Res. 21:375, 1942; Ehrenhaus, Nat. Conv. of I.A.D.R., March, 1940). Several investigators into the nature of "fusospirochetal" infections have used guinea pig lesions as a basis for study (Rosebury et al, J. Inf. Dis. 87:217, 1950; Macdonald et al, J. Inf. Dis. 95:275, 1954 and 98:15, 1956).

In preliminary studies, it was observed that in "Levaditi stained" sections of "Fusospirochetal" lesions in guinea pigs, a specific location of spirochetes and fusiforms could only occasionally be noticed. Furthermore, numerous structures resembling spirochetes could be seen in some of the lesions made with the four pathogenic components of a transmissible "fusospirochetal" infection described by Macdonald et al, 1956. Yet this combination did not include spirochetes. It is believed that this discrepancy warrants a further investigation of "fusospirochetal" lesions.

The objective of the present investigation is:

- (1) To study the distribution of component organisms of the "fusospirochetal" exudate HG (Macdonald et al, 1954) in guinea pig lesions using fluorescein tagged antibodies and histochemical stains on frozen fresh sections.
- (2) To study sections of human periodontal lesions using the same tagged antibodies and specifically tagged antibodies against pooled strains of oral spirochetes.

The experiments to date concern the four pathogenic components of exudate HG and spirochetes.

The organisms are:

a strain of a motile bacteroides	further designated as	K80
a strain of Bacteroides	"	" K92
a strain of Bacteroides negrescens	"	" K110
a strain of a facultative diphteroid	"	" JR3

The spirochetes included:

a strain isolated from exudate HG	"	"	" SHG
2 strains " " oral scrapings	"	"	" S ₁ & S ₂

During the 1958-59 tenure of grant DA-71, the following results were obtained:

(1) Gamma globulin, separated from sera of rabbits immunized with K80 organisms, was successfully tagged with fluorescein isothiocyanate.

(2) Spontaneous agglutination in antigen of K92 organisms, which interfered with the determination of the titer of antisera needed as a check on tagging procedures, could be prevented by using 0.1 M phosphate buffered saline at pH 7.2 in washing and diluting procedures.

(3) a. Haemoglobin was found to be a necessary requirement for growth of K110 organisms in liquid media. This substance, however, induced autolysis particularly in agar free media. The amount of 9 micrograms sheep's haemoglobin per cc of medium produced satisfactory heavy growth in six days without inducing perceptible autolysis in this time interval.

b. A growth factor produced by a strain of staphylococcus aureus or by strain JR3 could be successfully added in the form of a piece of agar cut from a plate next to growth of these organisms or as an ether extract of growth of these organisms in peptone water.

(4) The adjusting of potential, as the result of growing a gram + facultative anaerobic rod together with spirochetes in agar free 1 per cent ascitic fluid media incubated in a Nitrogen atmosphere, produced good growth of spirochetes. In serial subcultures in non ascitic fluid containing media, growth of the spirochetes progressively failed while the organism changed morphologically. Promising results were obtained when attempts were made to duplicate the potential in ascitic fluid containing agar free media.

(5) A method was developed to maintain a temperature between minus 4 and minus 2 degrees centigrade in an insulated chamber which permitted sectioning of frozen fresh tissues.

Experiments in 1959-60Experiments with K80

Using the tagged globulins prepared in 1958-59, slide preparations of K80 organisms could be satisfactorily stained following the methods described by Thomason et al, J. Bact. 74:525, 1957. The illumination used consisted of an arc lamp with Wratten 18A filter.

Experiments with K110

Several attempts were made to make antigens from heavy growth of K110 organisms in Difco Thioglycollate broth without agar and to which was added sheep's hemoglobin and an ether extract of JR3 organisms grown in peptone water in concentrations per cc of 9 micrograms and 0.05 cc respectively. However, these antigens were not satisfactory as heavy clumping of organisms occurred. The addition of Tween's, or buffering the saline at various pH's for washing purposes, failed to prevent clumping during preparation or after storing. It is assumed that this is the result of partial autolysis which is probably not perceptible in darkfield observation of the growth. In order to prevent or delay autolysis, it was attempted to standardize the growth of K110 cultures. As the growth factor was an unknown substance, experiments were made to standardize the methods for preparation. Both the staphylococcus and JR3 strain were used as producers. Two media were used; 1 per cent peptone water and heart infusion broth. (Difco B38) Inocula were standardized (1/10 ml of 24 hour broth culture). The effect of the following additions and manipulations upon the production and preparation of the growth factor were tested.

- (1) Addition of glucose (0.5 - 1 per cent) and thioglycollate singly and in combination.
- (2) Incubation for 24, 48, and 72 hours.
- (3) Extraction with ether (10, 20 and 30 per cent by volume) during 1 and 2 hours.
- (4) Evaporation of the ether under vacuum or in air.
- (5) Redissolving the residue in saline or saline alcohol mixture.
- (6) Combinations of the above.

The growth factor solutions obtained with these methods were tested each time in six fold, using 3 concentrations of addition (0.05, 0.1, 0.3 cc), for their effect upon the rate of growth of K110 organisms (density measurements). These trials failed to produce standardized growth of K110. The effect of

aging upon the extracts was investigated and it was found that in general, extracts could be stored in the dark at -4°C for three weeks, after which they deteriorated slowly.

It was learned at this point that the Bacteriological laboratory of the Forsyth Dental Infirmary had found that ether extracted growth factor could be substituted with Vitamin K or menadione. The substitution of menadione (.6 microgram/cc) for ether extracts in the basic medium produced reliable standardized growth of K110. It was found that, by avoiding maximum growth to develop through (1) reducing the incubation time to 4 days and (2) reducing the amount of sheep's haemoglobin to 6 micrograms/cc as well as heating the suspension of organisms to 60° for 30 minutes, well dispersed stable antigens could be produced.

It is felt that the production of stable well dispersed antigen was a necessary step in order to have a check on the agglutination titer of antisera during tagging procedures. Antisera have been produced and purification and tagging procedures are being performed.

Experiments with K92 and JR3

Well dispersed antigens were prepared of K92 organisms using the results obtained in 1958-59. Antigens of JR3 organisms were made from growth of organisms in heart infusion broth following standard methods. Antisera with a satisfactory agglutinating titer (1:2560) were obtained from rabbits immunized following the schedule used with K80 organisms. Purification and tagging procedures were undertaken.

At this time, the Division of Dental Research moved to new quarters at 124 Edward Street resulting in a temporary suspension of experimental work.

On resuming the experiments, it was found that precipitates had formed in the globulin solutions, probably due to a drop of the pH,, and the solutions had to be discarded. This drop in pH probably resulted from instability of the carbonate buffers used (recommended by Marshal, Proc. Soc. Exptl. Biol. and Med. 12:898, 1958). Since the use of tagged globulins from the same batch as those used for staining inhibition and background staining is recommended in the literature, new antisera had to be obtained.

This unfortunate occurrence, coupled with the unavoidable time lost in moving has been a severe hindrance in the progress of the project. It otherwise would have been well into the stage of staining guinea pig lesions made with the four pathogenic component organisms of HG.

Experiments with Spirochetes

In 1958-59, growth of spirochetes was obtained in two serial subcultures in agar free Proske and Sayers medium containing 1 per cent ascitic fluid. Upon further serial subculturing, growth progressively failed. Antigens made from these cultures were not satisfactory as large mucoid like masses of organisms were present in the suspensions. This was probably due to "carry-over" of agar or ascitic fluid. The total or partial substitution of ascitic fluid by sheep's serum, yeast extract, egg white, starch or gelatine in various concentrations and combinations did not support growth in agar free media beyond three or four serial subcultures. Media not containing at least 1 per cent ascitic fluid or serum would not support growth beyond one subculture. As it was noted that these non agar media were not well "poised", and that a successful subculture depended also on the speed of inoculation and reduction, it was attempted (1) to increase the poisoning capacity of the Proske and Sayers base by maintaining the pH at higher levels than prescribed in the recipe and (2) to obtain a lower potential by the addition of sugars to the media which had been reduced prior to inoculation. (Madlener, Proc. Nat. Conv. I.A.D.R., 1956). When the pH was maintained between 7.6 and 8.6, a medium was obtained with a much improved poisoning capacity (measured in time of oxidation of OX-red. indicator). To avoid uncontrollable single electrode potentials which complicate potentiometric measurements, it was decided to assess the influence of sugar additions directly on growth of spirochetes. Glucose, maltose and sucrose, sterilized either by autoclaving or filtering, were added singly and in combinations in 1 per cent concentration to non ascitic fluid, non agar containing base medium. These media were inoculated after reduction with a standard inoculum from growth in 1 per cent ascitic fluid and agar containing media. It was found that the addition of a combination of autoclaved glucose and Seitzed maltose supported growth longest (4 times) in serial subcultures. When a combination of these sugars was used in agar and ascitic fluid containing media, growth of spirochetes was markedly improved (from $20 \times 10^7/\text{cc}$ to $50 \times 10^7/\text{cc}$). Only a slight improvement of growth could be noticed in agar free media. The base medium of Proske and Sayers (Pub. Health Rep. 48:839, 1934) contains agar. The agar free media is obtained by filtration (Seitz) of the base. As this filtering process may also remove other substances in addition to agar, the method of preparation was modified by totally deleting agar. In this agar free base medium to which was added by volume 1 per cent autoclaved glucose, 1 per cent Seitzed maltose, 0.05 per cent thioglycollate and 5-10 per cent ascitic fluid, 30 serial subcultures of spirochetes have been made successfully, yielding $50-60 \times 10^7$ organisms/cc.

Thus it was found:

- (1) Spirochetes are weak acid producers (part of 1 unit).

(2) Growth occurred between pH of 6.7 and 9. The optimum for strains S₁ and S₂ was pH 7.6 and optimum for strain SHG was pH 8.2

(3) Actively metabolising spirochetes have reducing power, as shown in the following comparison of the time interval, following opening of a Brewer jar, in which total oxidation of the OX-red. indicator in the media occurred.

non inoculated tubes	10 min.
after 4 and 6 day's growth	45 min.
after 9 day's growth	30 min.
after 12 day's growth	15 min.

Optimum density of growth is usually attained after 6 days, 50-60 x 10⁷ organisms per cc.

(4) Serial subcultures in ascitic fluid free media failed after 3-4 times. However, growth in the third subculture seemed to be sufficient for antigen production. The ascitic fluid "carry-over" was calculated 0.04 per cent per cc.

Summary

(1) Slide preparations of K80 organisms have been successfully stained with tagged globulins.

(2) In antigen suspensions of K110 organisms, clumping, which occurred during preparation or after storage, could be prevented by a better controlled growth.

(3) Antisera against K92 and JR3 organisms have been obtained and purification and tagging procedures were undertaken.

(4) By modifying the method of preparing Proske and Sayers base medium in addition to adjusting the potential, over 30 serial subcultures of spirochetes in agar free media have been made. It was found that (a) spirochetes were weak sugar fermenters, (2) growth could be obtained at pH of 6.7 to 9, and the optimum pH for growth of the three strains was found, (c) actively metabolizing spirochetes possessed considerable reducing power. Growth in the third subculture in agar and ascitic fluid free media was promising for antigen production.

(5) Rapidly frozen biopsies from human periodontal lesions have been collected for future studies.

APPENDIX H

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Subject: Penetration of minerals from saliva and shifting of minerals in the carious lesion.

Grantee: Z. Mezl

Project: D.A. 79 Amount Awarded: \$2,000.00 - 1959-60

In May and June of 1959, I received, set up and tested, the Ultropak microscope and the accessories.

To confirm the reliability of the observations with the Ultropak, I studied over 50 initial carious lesions of enamel with combined methods: observation in reflected light cross-checked on the usual ground sections examined in transmitted and polarized light, with or without staining, and micro-hardness tests. The fundamental observations with Ultropak agree with the reports in the recent literature and the results of other studies. This was further confirmed by our observations on some lesions produced in vitro. The student assistant, Mr. Boily, helped in this part of the work; he prepared many ground sections, studied and tested special staining methods and duplicated experiments found in the literature.

I then studied the effects of demineralisation and remineralisation on "cytological level" as they appear in different parts of the rods, in the rod sheaths and in the interprismatic substance. This can be done in cross sections cut perpendicular to the rods and examined with Ultropak. I distinguished the earliest stages of demineralisation from hypocalcifications of developmental origin. I distinguished and described several types of remineralisation. The remineralisation may be selective, involving certain structures:

1. the enamel rods,
2. certain rods only,
3. lamellae or cracks,
4. It may be diffuse, and not limited to any particular structure.

Remineralisation is gradual and several stages can be distinguished. The histological appearance of remineralisation depends on the degree to which the area had been demineralised before the remineralisation started and on the stage reached by the remineralisation itself.

To distinguish the role of minerals coming from other areas of the lesion and those coming from outside, from saliva, I am now studying how different solutions penetrate into the various zones of the lesion. First, on selected lesions, I observe the penetration of stains from the surface through the pathways mentioned above. I suggested a classification of carious lesions based on their evolution, determined from the relations of demineralised and remineralised areas:

1. Progressive caries evolves from the initial demineralisation directly to destruction.
2. Alternating caries shows progress of demineralisation in some areas, progress of remineralisation in other areas.
3. Regressive caries shows wide areas of remineralisation, no progress of demineralisation. The remineralisation is not complete, demineralised areas without evidence of remineralisation are present.
4. Arrested caries, strictly speaking, are only lesions that have been entirely remineralised to a high degree. (Theoretically, this fact should be confirmed by serial sections.) More common are lesions with a satisfactorily remineralised surface layer, with the deeper parts showing remineralisation everywhere on the periphery, with small areas inside of the lesion showing relatively little remineralisation.

The practicability of this classification is being tested through observation on a large number of carious teeth.

Until now I have studied a little over 250 carious lesions. During the academic year, the Faculty increased my teaching program, and therefore less time is left for research work than was originally anticipated. The work is about two months behind the anticipated schedule and will be resumed with greater intensity following the spring term.

APPENDIX I

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Subject: Investigations into the nature of cementum.

Grantee: K. J. Paynter

Project: D.A. 64 Amount Awarded: \$3,000.00 - 1956-57
3,400.00 - 1957-58
2,000.00 - 1958-59
750.00 - 1959-60

Summary

The technique for obtaining thin (4 micron) sections of the mineralized dental tissues together with adjacent soft tissues, has been finally developed and is proving to be quite satisfactory.

Pilot studies involving the use of Tritium-labelled Thymidine to study odontogenesis have been completed, and further work evolving from the preliminary work is being planned.

Studies combining radioautography and biochemical analysis are being conducted to determine the form in which radioactive sulphur injected as labelled methionine finally becomes incorporated into the dental tissues.

No further funds will be necessary to support the continuance of this project at this time.

Body of Report

1. Method of Sectioning

We have found that when the plastic materials routinely used for embedding biological tissues (e.g. Ward's bioplastic, butyl and methyl methacrylate as employed for sectioning for the electron microscope) were polymerized sufficiently to hold dentine and enamel for sectioning, they became insoluble in all routine laboratory reagents and could be removed from the sections. Recently we have tried a plastic called Styrene ($\text{CH}_2=\text{CH}(\text{C}_6\text{H}_5)$) available from Eastman Organic Chemicals, which, after purification, will polymerize sufficiently hard, can be sectioned without fracturing at 4 microns, and remains readily soluble in common laboratory reagents such as acetone or xylene.

We have also found that utilizing the freeze-drying technique for dehydration and embedding ensures maximum impregnation of

plastic into the tissues. We have used a De-virtis Histo-freeze dryer for dehydration, and embedded the tissues while they were still under negative pressure. Using this method we have satisfactorily embedded the portion of 60 - 90 day old rat mandible containing the molar teeth and sectioned them at 4 microns longitudinally. Sections contain all the tissues including enamel.

This technique will now be used in further isotope studies using phosphorous and calcium so that the water-soluble components as well as the bound elements may be preserved in situ.

2. Preliminary work using Tritium-labelled Thymidine injected into both young rats and guinea pigs has led to some conjecture concerning the origin of ameloblast cells. Classically these cells are thought to arise from relatively undifferentiated cells at the area of the cervical loop of the enamel organ and to differentiate into the columnar form of ameloblast as they come to lie further up the inside of the crown of the tooth.

Our observations suggest that ameloblasts may arise in situ even some distance up the crown of the tooth, from cells of the stratum intermedium. Should this be so, it would indicate that ameloblast differentiation may extend over a considerably longer period of time than was formerly believed.

It is also quite evident that odontoblast cells do not divide themselves but arise from cells of the primitive pulp in the area of the open apical foramen.

3. Using S35-labelled methionine injected into rats, attempts are also under way to combine the localization of the activity using radioautography with a more precise localization chemically. The latter is being done by separating the enamel and dentine, and further breaking them down into their organic and inorganic components. We shall then find out in which component the activity becomes deposited.

A series of young rats have been injected with S35 labelled methionine. One quadrant of the jaws has been set aside for preparation of radioautographs. The remaining teeth have been powdered and a measure made of the total radioactivity in the powder. The tissues have been subsequently separated into enamel and dentine, and each of these has been broken down to its organic and inorganic components. The organic portion of each has further been sub-divided as to its solubility. This work is still under way and to date the results are by no means complete. It does appear, however, that the difference between activity in the total powdered specimens as compared to that found in the insoluble organic components indicates that probably most of the activity is contained either with the inorganic component or with the soluble organic fraction of the teeth.

It should be mentioned that the work involving radioautography is being done largely by Dr. A. M. Hunt, and that the work involving the breakdown of the dental tissues into their various components is done with the collaboration of Dr. G. Nikiforuk.

4. Since this work can be maintained throughout the coming year without additional funds, no further request for money is being submitted at this time.

Grantee: S. G. Perrineau and W. B. Donohue

Project: D.A. 60

Amount Awarded: \$4,000.00 - 1953-54

The purpose of this study is to observe the effects of massive dose of roentgen rays on the developing skull of young kittens.

An attempt was made as far as possible to use animals approximately three months of age. Because, at this age, the permanent dentition is in an active stage of development. From the pilot studies, it was evident that a low percentage of mortality was to be expected. If animals younger than three months of age were used.

Before any of the animals were radiated, their stage of dental development was checked by lateral skull x-rays. It was felt that the degree of dental development would serve as a rough check on the stage of facial and cranial growth reached by the animals at the time of radiation.

The mortality rate in both the treated and control animals was high during the first few weeks of the experiment. It was then evident that vaccination against feline distemper and feline parvovirus, as well as isolation from one another, and from the colony, during the period of "immunity", was essential if the animals were to survive for the required length of time (1 year).

Furthermore, the animals were quite susceptible to the high temperatures prevailing during the summer months; this delayed progress of the work until the cool weather arrived.

From the results observed in the pilot group of animals, that were radiated in the spring of 1954, the following physical radiation factors were decided upon: 210 K.V., 10 M.A., 1 cm. Al (Filtration), 21 cm. Target to skin distance. The total dose delivered was 3000 R per animal, divided into 1000 R to each side of the skull. This radiation was given in two sessions, one week apart.

APPENDIX J

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1960

Subject: The effects of a heavy dose of roentgen rays on the developing structures of the skull in young kittens.

Grantee: J. G. Perreault and W. B. Donohue

Project: D.A. 80

Amount Awarded: \$4,000.00 - 1959-60

The purpose of this study is to observe the effects of a massive dose of roentgen rays on the developing skull of young kittens.

An attempt was made as far as possible to use animals of approximately three months of age. Because, at this age, the permanent dentition is in an active stage of development. From the pilot studies, it was evident that a low percentage of survivals was to be expected if animals younger than three months of age were used.

Before any of the animals were radiated, their stage of dental development was checked by lateral skull x-rays. It was felt that the degree of dental development would serve as a rough check on the stage of facial and cranial growth reached by the animals at the time of radiation.

The mortality rate in both the treated and control animals was high during the first few months of the experiment. It was soon evident that vaccination against feline distemper and feline pneumonitis, as well as isolation from one another, and from the colony, during the period of "immunization", was essential if the animals were to survive for the required length of time (1 year).

Furthermore, the animals were quite susceptible to the high temperatures prevailing during the summer months; this delayed progress of the work until the cool weather arrived.

From the results observed in the pilot group of animals, that were radiated in the spring of 1959, the following physical radiation factors were decided upon; 110 K.V., 10 M.A., 3 mm. Al (Filtration), 21 cm. Target skin distance. The total dose delivered was 2000 R per animal, divided into 1000 R to each side of the skull. This radiation was given in two sessions, one week apart.

Initial Findings

Within one month following the experimental procedure, the usual reactions to radiation were noted: loss of hair, in some instances clouding of the cornea, weight loss, and a suppurative reaction in the nasal and oral passages of some animals. The animals studied to date were sacrificed after a post-radiation period of one month. They showed little pertinent tissue reaction histologically, and the serum alkaline phosphatase did not vary appreciably from the controls. However the number of animals studied so far is too small to arrive at any conclusions. Animals with longer survival periods have not yet been sacrificed.

Project: D.A. 80 Amount Awarded: \$4,000.00 - 1959-60

The purpose of this study is to observe the effects of a massive dose of roentgen rays on the developing skull of young kittens.

An attempt was made as far as possible to use animals of approximately three months of age. Because, at this age, the permanent dentition is in an active stage of development. From the pilot studies, it was evident that a low percentage of animals was to be expected if animals younger than three months of age were used.

Before any of the animals were radiated, their stage of dental development was checked by lateral skull x-rays. It was felt that the degree of dental development would serve as a rough check on the stage of facial and cranial growth reached by the animals at the time of radiation.

The mortality rate in both the treated and control animals was high during the first few months of the experiment. It was soon evident that vaccination against feline distemper and feline panleukopenia, as well as isolation from one another, and from the colony, during the period of "immunity", was essential if the animals were to survive for the required length of time (1 year).

Furthermore, the animals were quite susceptible to the high temperatures prevailing during the summer months; this delayed progress of the work until the cool weather arrived.

From the results observed in the pilot group of animals, that were radiated in the spring of 1959, the following physical radiation factors were decided upon: 110 K.V., 10 M.A., 3 mm. Al (Filter), 21 cm. Target skin distance. The total dose delivered was 2000 R per animal, divided into 1000 R on each side of the skull. This radiation was given in two sessions, one week apart.

APPENDIX K

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH NATIONAL RESEARCH COUNCIL

January 1960

Subject: (a) Secretion of bicarbonate ions in the saliva.
(b) Changes in the secretion of the developing salivary gland.

Grantee: A. S. V. Burgen

Project: D.T. 61 Amount Awarded: \$3,400.00 - 1955-56
3,400.00 - 1956-57
5,930.00 - 1957-58
5,050.00 - 1958-59
6,000.00 - 1959-60
6,000.00 - 1960-61
6,000.00 - 1961-62

(a) Numerous studies in the literature have shown only confusing relationships between changes in the plasma bicarbonate concentration and that in the saliva. Our own results were equally irregular. Sometimes an increase in serum bicarbonate caused a proportionate increase in saliva bicarbonate and sometimes no change at all. It has been found this year that perfectly regular results can be obtained provided that the pCO_2 of the plasma is controlled. An increase of arterial pCO_2 (caused by inhalation of high CO_2 mixture) increases the saliva/plasma ratio of bicarbonate; a decrease of arterial pCO_2 produced by hyperventilation decreases the saliva/plasma ratio. At a fixed pCO_2 the saliva/plasma ratio is fixed and independent of the absolute plasma bicarbonate. These relationships are quite predictable.

Alteration of pCO_2 provides a very useful method of altering the saliva bicarbonate which can be used to find out which ion changes are associated with it. Except when the saliva bicarbonate exceeds about 80 mEq/L changes in saliva bicarbonate are accompanied by equal and opposite changes in saliva chloride. At higher bicarbonate levels further increase in saliva bicarbonate is associated with a simultaneous increase in sodium. The reciprocity between bicarbonate and chloride suggests the presence of an anion exchange process. Further evidence suggestive of this has been obtained by the technique of close arterial injection of isotopes into the circulation of the gland. A distal permeability site has been found across which bicarbonate and chloride can exchange but across which cations cannot pass. Further experiments in this system are being carried out.

(b) In the newborn puppy, the salivary glands are in a very primitive state of development, there are practically no acini and

large dilated tubules. Despite this saliva is secreted well at least from four days of age. The saliva is less hypotonic than adult saliva and practically free of bicarbonate.

Hypotonicity steadily develops during the first three weeks and gradually the bicarbonate level rises towards the adult level. A correlation with the development of histological maturity is being studied.

Mrs. A. Wechsler obtained her M.Sc. degree for a thesis entitled "The Secretion of Bicarbonate in Saliva". She is proceeding to her Ph.D.

Dr. K. Martin is also working on problems of salivary secretion for his Ph.D.

The monograph on "The Physiology of Salivary Secretion" by Dr. Emmelin and myself is complete and ready to go to the publisher.

APPENDIX L

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH NATIONAL RESEARCH COUNCIL

January 1960

Subject: Hormonal factors and their relationships of citrate metabolism in bones and teeth.

Grantee: Jean-Paul Lussier

Project: No. D.T. 70

Amount Awarded: \$6360.00 - 1958-59
4800.00 - 1959-60
4800.00 - 1960-61
4800.00 - 1961-62

Introduction

The presence of citrate in bone and its production at this site could lead to thinking that we are dealing with a local mechanism until someone finds that factors of variation influencing this particular tissue are also producing measurable effects in other tissues and systems. For instance vitamin D is known to stimulate citrate production in bone (1). It has also been shown that citrate, under these conditions, is also increased in most tissues, except liver (2). The parathyroid hormone (PTH) which can also increase the citrate content in bone has not been much studied with respect to its extraosseous effects.

It has been intended in our experiments to measure some parameters which could bring information on the extent of changes connected with the production or utilization of citrate in the whole body. The three following parameters were measured after rats were injected with radioactive citric acid:

- a) Radioactive respiratory CO_2 which reflects the rate of utilization of citric acid.
- b) The presence in the urine of citric acid after intermediaries which reflect the rate of excretion and the sites of inhibition of stimulation.
- c) The presence of radioactive acid and radiocarbonate in bone which give information about the available size of the skeleton and may be used as a standard of reference.

The present report gives mention of the results collected from experiments performed in accordance with this hypothesis.

I. Influence of the parathyroid hormone on citric acid metabolism. Synergism with fluoroacetate.

Methods

Methods and procedures have been described in a previous report (3). One point is worth of noting which last year has been left unsolved. After bone fragments have been used for CO₂ determination, the remaining sulfuric solution contains radiocitric acid. It has to be processed in such a way as to be evaporated or concentrated for better radioactive measurements and chromatographic identifications. In order to put up a technic which would serve these purposes, the following procedure was proposed.

A solution of H₂SO₄ 20% was prepared which also contained small amounts of radiocitric acid. The radioactivity of the whole solution was 974 cpm/ml. An aliquots of this solution was mixed with a specific solvent to be in a separating funnel. After one minute of vigorous shaking and clear separation of the two layers, an aliquot of the solvent was taken, evaporated and counted. Table I shows the results obtained with different solvents used in various proportions.

Table I

The addition of isoamyl alcohol, isopropyl alcohol or methyl cellosolve made the separation less sharp, without adding to the extracting power of the solvent. Also neutralizing the solution (partially or completely) the extracting power of the solvent was decreased rather than improved. It is found from our data that even if ethyl acetate can extract up to 68% after one minute of contact when 10 parts of the solvent are used with one part of the sulfuric solution, it is not enough to ascertain reliable and reproducible results. We are actually working with a liquid-liquid extraction apparatus which operates continuously. We expect by using longer periods and heated solvent that the extraction of citric acid will be quantitative.

TABLE I

<u>Solvent</u>	<u>Volume</u>	<u>Volume of</u> <u>Sulfuric * Sol.</u>	<u>% extracted</u>	<u>Time (min.)</u>
Ethyl acetate	5	1	22.5	1
"	5	1	28	2
"	5	2.5	8	1
"	5	2.5	19	2
"	10	1	68	1
"	10	1	64	2
Amyl acetate	10	1	14	1
"	10	1	15	2
Ethyl acetate	5	1	40	1
Amyl acetate	5		52	2

* containing radioactive citric acid.

Results

From the data found in Table II some indications can be found which suggest a broad action of PTH upon citric utilization. It should be mentioned right now that we depend on averages without standard errors as the number of individuals is often insufficient to justify statistical analyses. When the groups will be completed to 7 animals each, statistical analyses will then be done. The administration of 50 units of PTH reflects upon the amount of respiratory CO_2 exhaled and upon the amount of radioactivity therein contained. The administration of 2.5 mg/kg of fluoroacetate produces an effect similar to the one caused by PTH. When both compounds (PTH and fluoroacetate 5 mg/kg) are used, this combination produces a greater effect. As a matter of fact, it appears that PTH has a slight inhibiting effect on respiration. The administration of 5.0 mg/kg of fluoroacetate decreases very severely the respiratory CO_2 down to 0.66 gr/hr. The same amount of fluoroacetate plus 15 units of PTH decreases it further down to 0.44 gr/hr. The radioactivity contained in this respiratory CO_2 follows the same trend. The impact of this inhibition is affecting very seriously the citrate oxidation as less than 10% of the normal value is found in the last group. It cannot be said at this moment that PTH is acting specifically on citrogenase as it is the case for fluoroacetate. The decreased CO_2 in the respiratory gases could be the result of a secondary reaction to acidosis produced by the loss of phosphates through the urine.

The content of carbonate in bone does not differ appreciably from one group to another. However, the specific activity of carbonate (radio CO_2 cpm/ CO_2 mg) has been influenced differently by fluoroacetate. At the rate of 2.5 mg/kg, there seems to be a slight increase of radio CO_2 in bone over the normal value; these values however are not too dependable as only two animals were treated in this manner. At the rate of 5.0 mg/kg, it is found that the radioactivity in the carbonate fraction is markedly reduced. This is explainable by the fact that citric acid is less utilized, therefore less radioactive CO_2 is transported in the blood and thus enters into the bone. It is interesting to see that PTH acts also likewise. The combination of both factors makes the bone almost free from radioactive CO_2 . The fact that PTH potentiates the inhibiting effect of fluoroacetate suggests that PTH may have a site of action different from the citrogenase system.

The specific activity of bone citric acid could not be determined at this moment. It can be shown however that the content of citric acid in bone is decreased following the addition of PTH which is not case of fluoroacetate. This indication has to be substantiated by more data.

It can be concluded from this still incomplete work that PTH and fluoroacetate have some similarities in their effect on citrate metabolism as there is: a) a decrease on respiration,

b) a decrease on citric acid utilizations, and c) a decrease of the circulating bicarbonate. The fact that PTH in combination to fluoroacetate is more efficient than each one done may be that they are acting at different rites. For PTH this site may be the bones, such may not be the case for fluoroacetate.

TABLE II

Methods

Nephrectomized male rats were injected 24 hours after the operation with 1 ml of radioactive citric acid and immediately placed in a metabolic cage for a period of one to six hours.

Hepatectomized rats were injected 24 to 48 hours after the operation with 1 ml of radiocitric acid as in the previous group.

Nephrectomized-hepatectomized rats had the liver removed two days before the kidneys. Injection was given on the day following nephrectomy. Normal rats were injected likewise and served as controls.

After the observation period, they were sacrificed. Bone of the hind limbs were removed for carbonate and citrate analysis.

Results

The available data on this part are dealing with the radioactive respiratory CO_2 and bone radiocarbonate. Graph I shows the amount of radioactivity found in the respiratory CO_2 .

The hepatectomized animals are utilizing citrate more rapidly than the normals. It seems that an inhibitory factor is removed or that liver regeneration calls for more energy, thus increasing the rate of CHO metabolism.

The nephrectomized animals can be divided in two subgroups: those which follow the normal curve and those which are below. A way or another, it is quite indicative that nephrectomy does not impair very seriously the rate of citrate utilization and that other tissues are getting rid of it within a few hours. The difference between the two subgroups may come from the presence of alkalotic conditions. There are some indications that the production of respiratory carbonate is significantly decreased.

Surprisingly enough, hepatectomy instead of bringing the values of the nephrectomized animals back to normal, further decreases the ability of the organism to metabolize citric acid.

TABLE II

Influence of PTH on metabolism of citric acid

	<u>Respiratory CO₂</u>		<u>Urine</u> Radioactivity (% injection)	<u>Bone CO₂</u>			<u>Citric Acid</u> mg/100mg dry bone
	Rate (gr. of BaCO ₃ /hr)	Radioactivity (% injection)		mg/100mg dry bone	cpm/mgCO ₂	Radioactivity cpm/100mg dry bone	
<u>Normaux</u>							
1) N 3	1.30	32	5.2	1.74	50	80	0.55
<u>PTH</u>							
4) N 5	0.90	26	0.2	1.58	34.2	34.1	0.43
<u>Fl. Acet.</u>							
3) N 5 *	0.66	5.5	0.8	1.66	22.7	36.1	0.59
4) N 2 #	0.84	24.	5.5	1.56	68.1	102	0.61
<u>PTH</u> <u>Fl. Acet.</u>							
5) N 4	0.44	2.9	1.0	1.65	4.0	7.1	0.56

* These animals received 5 mg/kg of fluoroacetate

These animals received 2.5 mg/kg of fluoroacetate

However this effect is of short duration as after six hours the amount of radioactive CO_2 liberated has almost reached the normal level.

It is rather deceiving to observe that the so-called regulating mechanism of the kidney does not show more dramatically its importance under these circumstances. The only explanation is that the degradation of citric acid in the kidney does not go as far as CO_2 but may stop at the step of lactic acid. Therefore if the final steps (from lactic acid to carbon dioxide) are carried by the other tissues, it will not show much whether the kidney is present or not when only the final end-product is considered.

We have no way to explain the cumulative depressive effect of combined hepatectomy and nephrectomy. Metabolic and circulatory disorders may be such in these animals as to delay the transport of injected solution to the tissues and the reaching of the ultimate dynamic equilibria.

In Graph II are represented the specific activities of bone carbonate determined in the different groups. It is easier to discriminate a more specific effect of the kidney on citrate metabolism from these curves than from the previous graph.

The normal animals do not store radiocarbonate very long as a very small amount subsists after four hours. The hepatectomized animals are retaining a little more. This is not consistent with the data for the respiratory CO_2 . These data need statistical evaluation and more of them must then be gathered.

The nephrectomized, and hepatectomized-nephrectomized animals may be considered as a single group, as they retain the carbonate in bone at almost a constant level from one to six hours. If we take for granted this remark from Neuman and Neuman, that the concentration of a compound which does not enter the crystal lattice of bone is more reliable than its concentration in the blood, we can extrapolate to the point, that carbonate, although it can enter the space lattice, is observed here only a short time after its introduction in bone and may therefore be identified as being part of the rapidly exchangeable fraction of the bone mineral.

We may conclude that the metabolism of citrate may not be so much delayed after nephrectomy. But the circulation in bone as a result of this operation, becomes so sluggish that the changes therein noticed must be cautiously interpreted.

We are awaiting the analysis of citrate in bone to see if the specific activities of citric acid in these groups will behave like the carbonate ones.

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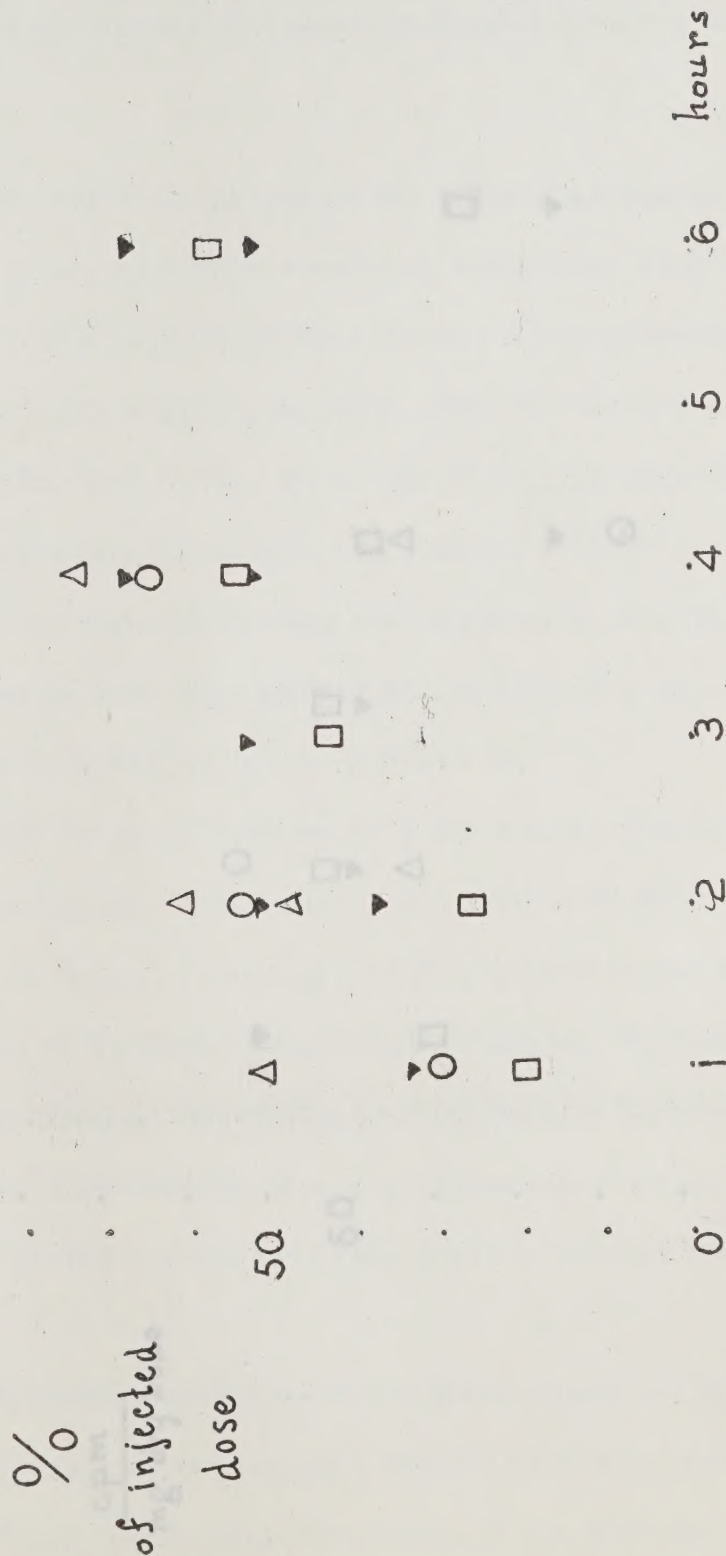
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GRAPH I

RADIOACTIVITY OF

RESPIRATORY CO₂

- O — NORMALS
- Δ — HEPATECTOMIZED
- ▼ — NEPHRECTOMIZED
- — NEPH-HEPATECTOMIZED

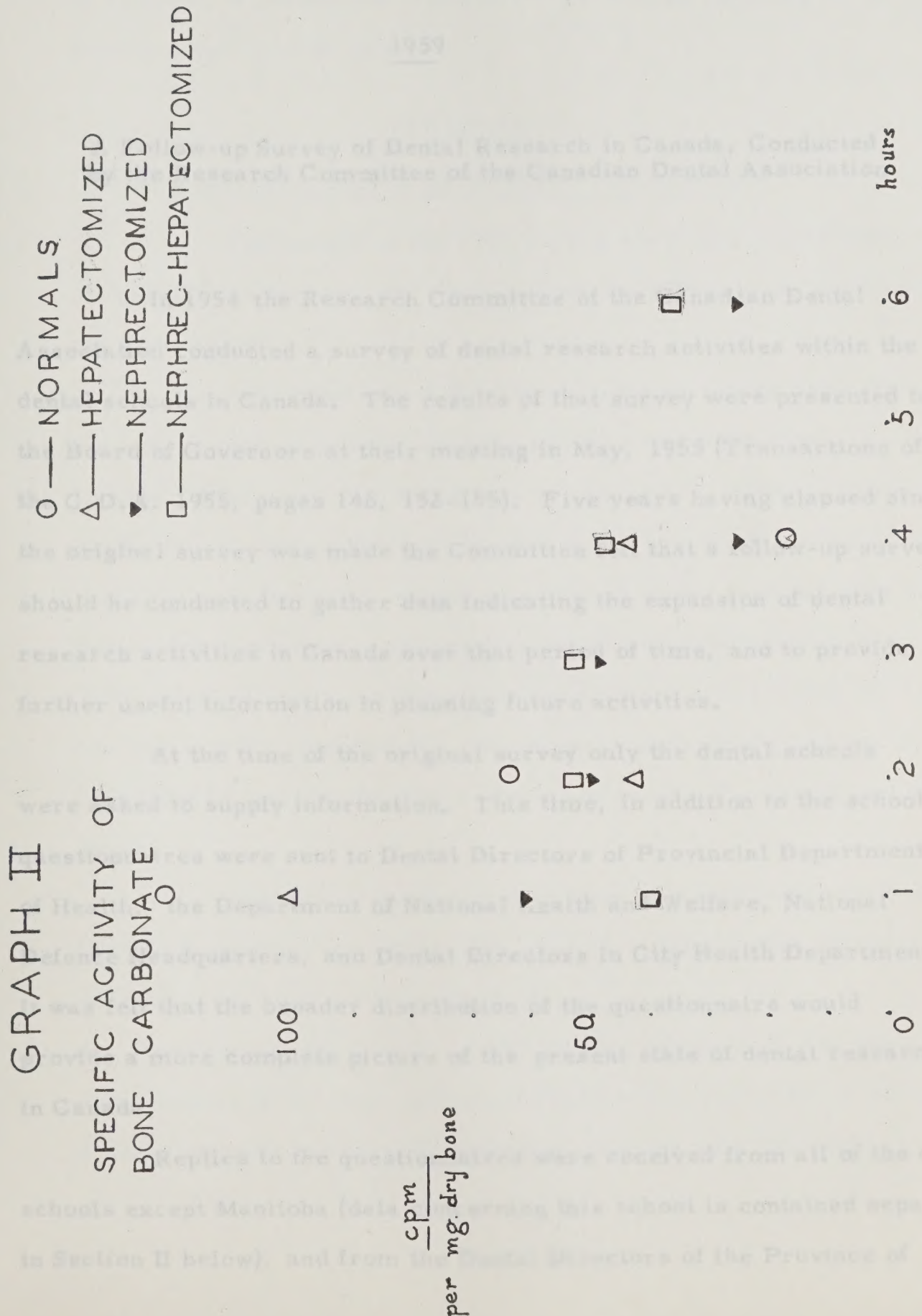


Each point represents two to four animals.

APPENDIX M

DENTAL RESEARCH IN CANADA

1959



APPENDIX M

DENTAL RESEARCH IN CANADA

1959

A Follow-up Survey of Dental Research in Canada, Conducted
by the Research Committee of the Canadian Dental Association

In 1954 the Research Committee of the Canadian Dental Association conducted a survey of dental research activities within the dental schools in Canada. The results of that survey were presented to the Board of Governors at their meeting in May, 1955 (Transactions of the C.D.A. 1955, pages 146, 152-155). Five years having elapsed since the original survey was made the Committee felt that a follow-up survey should be conducted to gather data indicating the expansion of dental research activities in Canada over that period of time, and to provide further useful information in planning future activities.

At the time of the original survey only the dental schools were asked to supply information. This time, in addition to the schools, questionnaires were sent to Dental Directors of Provincial Departments of Health, the Department of National Health and Welfare, National Defence Headquarters, and Dental Directors in City Health Departments. It was felt that the broader distribution of the questionnaire would provide a more complete picture of the present state of dental research in Canada.

Replies to the questionnaires were received from all of the dental schools except Manitoba (data concerning this school is contained separately in Section II below), and from the Dental Directors of the Province of

Prince Edward Island, Nova Scotia, New Brunswick, Saskatchewan and British Columbia, from the Directors of Dental Services for the Cities of Toronto, Winnipeg and Vancouver, as well as from the Royal Canadian Dental Corps (see Section IV).

RESULTS

I DENTAL SCHOOLS (EXCEPT MANITOBA)

The details of the 1959 survey are presented in Tables I - VI. To permit comparison, the figures from the 1954 survey are listed in the same table.

In general, the 1959 figures indicate a very dramatic and exciting change in both the quantity and type of dental research in Canada. Five years ago there was no research at all being conducted by the dental staff in three of the five dental schools in operation at that time; now however, all schools, including the newest, have active research programmes underway, being directed by dental personnel. The amount of space available for research activities within the schools has increased tremendously, - an increase undoubtedly related to the expansion in teaching facilities which has occurred over the last five years.

Comments related to each item on which data have been gathered in the survey are presented below, together with certain conclusions which may be inferred from the data.

A. Professional Personnel The figures (Table I) do not indicate much change in the total number of professional personnel in dental schools engaged in research activities over the past five years.

However the type of research personnel has changed, and this is of particular significance.

The number of people engaged in dental research who hold only the D.D.S. degree has decreased from an estimated ten in 1954 (33% of the total) to three at present (11% of the total). This decrease probably indicates a corresponding reduction in "short term" research projects such as have been conducted in the past by practicing dentists working on a part-time basis in research laboratories. At the same time the number of personnel engaged in dental research who hold both a dental and a basic science degree has increased from eleven (37% of the total) to twenty-one (75% of the total) over the past five years. The importance of this increase should not be overlooked, indicating as it does, that efforts to encourage young dental graduates to acquire basic science training with a view to becoming research workers and teachers have paid off handsomely. Furthermore, the fact that nearly twice as many people with special research training are now engaged in dental research as there were five years ago probably indicates a change in the type of research being conducted in the schools. Generally speaking dentists with additional graduate degree training, who are making a career of research and teaching, engage in long-term, basic investigations which, in the long run, pay much higher dividends than do the short-term projects.

The number of non-dental research workers in universities (who hold graduate degrees only) working on projects which might be considered dental research has decreased from nine to four. These

figures are among the least accurate of those collected because of the difficulty of collecting such data, and probably are below the actual number. Many University departments are engaged in research projects which, while they may be classed as non-dental, may well turn out to be highly significant to dentistry. The principles of biology apply to dental tissues as well as to any other.

B. Technical Personnel The actual number of technical personnel employed on research projects in dental schools has not increased over the last five years, although the number of "technical man-hours" has almost tripled (Table II). The amount of the research technician's time spent in research activities has increased from approximately thirty-three percent five years ago to almost one hundred per cent to-day, - another indication of the quantitative expansion in research which has occurred.

C. Graduate Students Most of the responsibility for training graduate students in the dental schools in Canada is still being carried by one school (Table III). However, recognizing that graduate instruction can only be adequately done where research is also being done, it is hoped that as the newer research programmes continue to grow and mature, other schools will ultimately assume their responsibility for training graduate students.

D. Facilities It is most gratifying to see that the space available for research activities in the Canadian dental schools has increased by a factor of approximately eight in the past five years (Table IV). The laboratories so provided are, in almost all instances, considered to be adequately equipped. It is also gratifying to note that all dental

schools continue to enjoy a happy and constructive relationship with basic science departments in other University faculties. Almost every survey reply indicated the willingness of these departments to share space and facilities with dental personnel interested in conducting research.

E. Salaries Five years ago it was indicated in the survey that a substantial amount of the salaries paid to professional personnel engaged in research in dental schools was provided by Grants-in-Aid. To-day virtually all professional salaries are being paid by the Universities, - a very practical demonstration of the willingness of the teaching institutions to assume their responsibility for supporting dental research.

Technical salaries, as might be expected, continue to be paid to a large extent, through Grants-in-Aid, available to all schools.

F. Annual Budget Because of the financial arrangements within the Universities it has not been possible to estimate what proportion of the annual dental school expenditure is allocated for the purpose of supporting research. However, the increase in space which has been provided in the schools and the increase in numbers of professional personnel being paid by the schools is indicative of the fact that this item has also increased considerably over the last five years.

Some indication of the amount of money being spent annually for dental research in Canada may be gained from a look at the figures published by the National Research Council of Canada, and the Canadian Dental Association (Table V). In 1954 the N.R.C. Associate Committee

on Dental Research allocated \$23,645 for Grants-in Aid, as direct support for research. In 1959 the corresponding figure was \$53,800, i.e. more than twice the amount.

Funds allocated by both the National Research Council and the Canadian Dental Association to support dentists while acquiring research training, which may be considered indirect research support, totalled \$10,108.00 in 1954, and \$30,107.00 in 1959. Of this latter figure nearly \$10,000 went to support undergraduate dental students working in research laboratories during the summer months.

G. Publications The number of publications of a research nature produced by an institution are simply another crude measure of the quantity of the research being conducted in that institution. Thus the Canadian dental school which has the largest programme of research has produced the most scientific publications (Table VI). The number from one of the other schools has very markedly increased, and more may be expected from others as their programmes expand.

II THE UNIVERSITY OF MANITOBA

No figures are immediately available indicating the amount of research presently being conducted at the University of Manitoba Faculty of Dentistry. This school is still in the process of being constructed. Nevertheless some significant information has been provided in a report by Dean Neilson to the Associate Committee on Dental Research of the National Research Council in March, 1959. Dean Neilson indicated that twelve or thirteen full-time clinical personnel and seven full-time basic science personnel would be employed by the dental school when it was completed. He expected that the

teaching load of each of these people would not exceed about 12 hours per week, - thus providing considerable time for the research projects.

Of the total floor space of 57,000 square feet in the new school in Manitoba, more than 10% (6300 square feet) has been set aside for research laboratories, with opportunities for future expansion should this become necessary.

It is worth noting that two National Research Council Grants-in-Aid were made to the Manitoba School in 1959 to support research projects already under way. In addition several young dental graduates are now away acquiring further training to equip themselves as teachers and research workers in Manitoba.

With such an auspicious beginning it is expected that the future holds great promise for research at the University of Manitoba dental school.

III DENTAL RESEARCH IN UNIVERSITY DEPARTMENTS

OUTSIDE DENTAL SCHOOLS

In addition to the projects being carried out within dental schools in Canada, University departments not located in the dental school or in Universities with no dental school also conduct research which is of keen interest and great importance to dentistry. By way of illustration one could point to the excellent work being conducted at the University of Ottawa, McGill University and the University of British Columbia on problems relating to the mineralization of hard tissues, and to that in Dalhousie University on dental pulp vascularity and on the metabolism of local anaesthetics.

IV ORGANIZATIONS OTHER THAN TEACHING INSTITUTIONS

Replies to the questionnaires sent to Directors of the Health Units in Canada indicate that considerable time is being spent by both the Directors and their staff in research activities. Several studies are now under way in the East and the West to evaluate topically-applied stannous fluoride in preventing dental decay. Subjects included in the studies range in age from pre-school children to adults. One study has been started to evaluate the use of sodium fluoride tablets as a caries preventive measure. In addition a great deal of effort is being spent in gathering data to serve as a most important "Index of Dental Health" in the various regions of Canada. Finally some limited work is going on to evaluate various treatment techniques within dental health units.

It is perhaps especially noteworthy that the Royal Canadian Dental Corps is supporting one of the more extensive clinical evaluations of topical stannous fluoride.

V SUMMARY

The results of the 1959 follow-up survey of dental research in Canada conducted by the C.D.A. Research Committee, indicate that there has been a very significant increase over the past five years in the amount of dental research being conducted in Canada.

There is also good reason to believe that the type of research project has changed, - that less short-term projects and more long-term basic types of programmes are in operation to-day.

The number of research-trained dental personnel employed in Canadian Dental Schools has almost doubled during the five year span.

It is well worth noting that the policy of providing Student-ships by the Canadian Dental Association which have assisted these men in acquiring the kind of training necessary to become a research worker, has paid such large and rewarding dividends.

The amount of space assigned for research activities in Canadian Dental Schools has increased along with the increase in teaching facilities, and the new laboratories thus provided are being equipped as fast as they are being constructed.

	(0)	2 (0)	3 (3)	30-50	Pathology Endodontics
	(0)	1 (0)	1 (3)	20-30	Anatomy Pharmacology Surgery Periodontics
	(0)	1 (0)	1 (3)	20-30	Histology Pharmacology
	1 (3)	3 (3)	(3)	20-30	Pathology Physiology
	(9)	32 (9)	1 (4)	25-100	Microbiology Biochemistry Statistics Radiation Biology Pathology Pharmacology Surgery Histology Genetics Orthodontics
	3 (0)	21 (3)	4 (9)		

January, 1960

TABLE IProfessional Personnel Engaged in Research in Canadian Dental Schools1959

School	D.D.S. Degree Only	D.D.S. and Basic Science Degrees	Basic Science Degree Only	% Time Devoted to Research	Field of Investigation
Alberta	2 (0)*	3 (0)	(2)	5-10%	Pedodontics Orthodontics Pathology Endodontics
Dalhousie	(0)	2 (0)	2 (1)	30-50	Anatomy Pharmacology Surgery Periodontics
McGill	(0)	1 (0)	1 (1)	20-30	Histology Pharmacology
Montreal	1 (1)	3 (2)	(1)	20-30	Pathology Physiology
Toronto	(9)	12 (9)	1 (4)	25-100	Histochemistry Biochemistry Statistics Radiation Biology
	3 (10)	21 (11)	4 (9)		Pathology Periodontics Bacteriology Histology Genetics Orthodontics

The numbers in the brackets are taken from the 1954 survey.

TABLE IITechnical Personnel Engaged in Research in Canadian Dental Schools1959

School	No.	% Time in Research
Alberta	1	25% (35)*
Dalhousie	1	100% (33)
McGill	1	100% (25)
Montreal	1 (0)	100%
Toronto	9 (10)	3:50% 6:100%
	<u>13</u>	

* The numbers in brackets are taken from the 1954 survey.

TABLE IIIGraduate Students, Canadian Dental Schools, 1959

School	No.	Course
Alberta	-	
Dalhousie	-	
McGill	-	
Montreal	-	
Toronto	6	Grad. Degrees in Anatomy Embryology Growth & Development Histology
	+	
	15	Post- Grad. in clinical specialty fields in which re- search plays a part

TABLE IVResearch Facilities, Canadian Dental Schools, 1959

School	Floor Space	Location	Are Labs in Dental School Adequately Equipped?	Is Equipment in Other Departments Available to Dental School?
Alberta	100 sq.ft. (0)* approx.	Dental School	No	Yes
	?	Edmonton Central Palsy Clinic		
	?	Health Units	?	
Dalhousie	1000-1200 ft. (0)	Dental School	Yes	Yes
	1000-1500 ft. approx	Depts. of Anatomy Biochem- istry Physiology Pharmacol- ogy		
McGill	230 sq. ft. (0)	Dental School	Yes	Yes
	?	Dept. of Anatomy Pharmacol- ogy		
Montreal	2000 (600)	Dental School	Not Sufficiently	Yes
Toronto	25000 (4000)	Dental School	Yes	Yes
	200 +	Hosp. for Sick Children		
	200	Best Inst.		

* The numbers in brackets are taken from the 1954 survey.

TABLE V

Allocations for Direct and Indirect Support
of Research by National Research Council and
the Canadian Dental Association, 1954 and 1959.

	<u>1954</u>	<u>1959</u>
National Research Council Allocations		
Grants-in-Aid	23,645	53,800
Fellowships	6,000	16,000
Summer Assistantships	Nil	6,755
Sub-Total	29,645	76,555
 Canadian Dental Association Allocations		
Studentships - Regular	3,660	3,900
Studentships - Summer	Nil	3,195
Travel	448	257
	4,108	7,352
 Total	<u>33,753</u>	<u>83,907</u>

NATIONAL RESEARCH COUNCIL

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

TABLE VI

Research Publications from Canadian Dental Schools

	<u>Before 1954</u>	<u>1954-59</u>
Alberta	2	0
Dalhousie	0	0
McGill	10	2
Montreal	3	12
Toronto	36	44

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APPENDIX N

NATIONAL RESEARCH COUNCIL

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

PUBLICATIONS ARISING OUT OF WORK SUPPORTED
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Manitoba

2nd

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J. R. Tread
J. W. Hallam

Dredgold, V. H.

18

Toronto

1st

G. S. Church
A. M. Hall
J. E. Anderson
G. W. Brown

Fletcher, R. G.

24

McGill

3rd

C. P. Latham

Goring, H.

19

McGill

3rd

H. G. Shaw

Griffin, D. S.

11

Toronto

3rd

J. E. Anderson
A. J. Tread
G. W. Brown

Jow, P.

24

Toronto

3rd

G. W. Brown
A. M. Hall
G. E. Colwell

APPENDIX O

Applications for Summer Undergraduates Dental Research Assistantships

<u>Name</u>	<u>Age</u>	<u>University</u>	<u>Year</u>	<u>Sponsors</u>
Abood, J. G.	27	McGill	2nd	J. D. Fenwick J. McCutcheon Z. B. Nyeste A. S. V. Burgen
Berube, M.	23	Montreal	2nd	J. P. Lachance G. Gauthier J. -P. Lussier J. Frappier
Booth, W. G.	22	Dalhousie	3rd	R. H. Bingham J. D. McLean A. E. Hoffman
Ching, L.	29	Manitoba	2nd	F. J. Marshall J. R. Trott J. W. Neilson
Claman, U. R.	24	Manitoba	2nd	F. J. Marshall J. R. Trott J. W. Neilson
Direnfeld, V. N	19	Toronto	1st	C. S. Churcher A. M. Hunt J. E. Anderson G. Nikiforuk
Fletcher, R. G.	26	McGill	3rd	C. P. Leblond
Goring, N.	30	McGill	3rd	H. G. Dion
Gratton, D. R.	21	Toronto	3rd	J. E. Anderson K. J. Paynter G. Nikiforuk
Jow, R.	24	Toronto	3rd	G. Nikiforuk A. M. Hunt G. E. Connell

Members of the Association Committee on Dental Research

<u>Name</u>	<u>Age</u>	<u>University</u>	<u>Year</u>	<u>Sponsors</u>
Kaufman, H. W.	19	Manitoba	1st	J. Doupe I. Kleinberg P. Hagen
Tabourian, G.	23	Montreal	1st	J. -P. Lussier J. M. Blais J. P. Lachance
Taichman, N. S.	23	Toronto	3rd	J. E. Anderson K. J. Paynter A. M. Hunt
Trester, P. H.	20	Manitoba	2nd	I. Kleinberg G. A. Brass J. R. Trott
Weinlander, G. H.	29	McGill	3rd	C. P. Leblond L. E. Francis J. McCutcheon
Yeung, E.	26	McGill	2nd	

Representing the Dental Profession

- Dr. C. Castaldi,
Faculty of Dentistry,
University of Alberta,
Edmonton, Alta.
- Dr. L. E. Francis,
Department of Clinical Dentistry,
McGill University,
Montreal, P. Q.
- Dr. I. Kleinberg,
Department of Biochemistry,
University of Manitoba,
Winnipeg, Man.

* Member of the Executive

APPENDIX P

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointments

	<u>Term to Expire</u>
* 1. Dr. K. J. Paynter, <u>Chairman</u> Division of Dental Research, University of Toronto, Toronto, Ont.	31 March, 1962
2. Dr. E. W. R. Steacie, <u>ex officio</u> , President, National Research Council, Ottawa 2, Ont.	- - -
3. Dr. R. F. Farquharson, <u>ex officio</u> , Vice-President (Medical), National Research Council, Ottawa 2, Ont.	- - -
4. Dr. D. L. Thomson, <u>ex officio</u> , Vice-Principal, McGill University, Montreal, P. Q.	- - -

Representing the Dental Profession

5. Dr. C. Castaldi, Faculty of Dentistry, University of Alberta, Edmonton, Alta.	31 March, 1960
6. Dr. L. E. Francis, Department of Clinical Dentistry, McGill University, Montreal, P. Q.	31 March, 1962
7. Dr. I. Kleinberg, Department of Biochemistry, University of Manitoba, Winnipeg, Man.	31 March, 1962

* Member of the Executive

Term to Expire

- | | | |
|-------|--|----------------|
| * 8. | Dr. Jean-Paul Lussier,
Faculty of Dentistry,
University of Montreal,
Montreal, P.Q. | 31 March, 1960 |
| 9. | Dr. J.W. Neilson,
Dean, Faculty of Dentistry,
University of Manitoba,
Winnipeg, Man. | 31 March, 1962 |
| * 10. | Dr. C.H.M. Williams,
Associate Professor of Periodontology,
Faculty of Dentistry,
University of Toronto,
Toronto 5, Ont. | 31 March, 1960 |

Representing the Royal Canadian Dental Corps,
Department of National Defence

- | | | |
|-----|---|-------|
| 11. | Brig. K.M. Baird, <u>ex officio</u> ,
Director General of Dental Service,
Royal Canadian Dental Corps,
Department of National Defence,
Ottawa, Ont. | - - - |
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Representing the Division of Dental Health,
Department of National Health and Welfare

- | | | |
|-----|---|-------|
| 12. | Dr. H.K. Brown, <u>ex officio</u> ,
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ont. | - - - |
|-----|---|-------|

Representing Dental Research,
Department of Veterans Affairs

- | | | |
|-----|--|-------|
| 13. | Dr. L.A. Kilburn, <u>ex officio</u> ,
Director of Dental Research,
Department of Veterans Affairs,
Ottawa, Ont. | - - - |
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* Member of Executive

Term to ExpireRepresenting the Basic and Medical Sciences

14. Dr. H. J. Barrie,
Department of Pathology,
University of Toronto,
Toronto 5, Ont. 31 March, 1960
15. Dr. L. F. Belanger,
Department of Histology,
University of Ottawa,
Ottawa, Ont. 31 March, 1962
- * 16. Dr. A. D'Iorio,
Department of Physiology,
University of Montreal,
Montreal, P.Q. 31 March, 1961
17. Dr. A. G. Gornall,
Department of Pathological Chemistry,
University of Toronto,
Toronto, Ont. 31 March, 1962
18. Dr. J. B. Macdonald,
Director,
Forsyth Dental Infirmary for Children,
140 The Fenway,
Boston 15, Mass. 31 March, 1960
19. Dr. R. L. deC. H. Saunders,
Department of Anatomy,
Dalhousie University,
Halifax, N.S. 31 March, 1961
- * 20. Dr. G. D. Scott,
Department of Physics,
University of Toronto,
Toronto 5, Ont. 31 March, 1960
21. Miss D. J. Wright, Secretary
National Research Council,
Ottawa, 2, Ont. - - -

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